Personality Traits and Substance Abuse - A Case/Control Association Study on Receptor Gene Polymorphisms in Chinese Psychostimulant Users

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ABSTRACT

The use of psychoactive substance like ketamine, 3,4-methylenedioxy-methamphetamine (MDMA/ 'ecstasy'), methamphetamine (METH/ 'ice') and marijuana among adolescents has become more popular in Hong Kong these past few years especially with a dramatic increase in ketamine use. To date, rave parties and discos are events where people usually take these recreational drugs.

It has been shown that there is a higher risk for substance abuse in people who have been characterized to have personality traits like novelty seeking and harm avoidance behaviours. These behaviours have also been shown to have an association with certain gene variants. These gene variants include catechol-*O*-methyltransferase (COMT), monoamine oxidase-A (MAO-A), human mu-opioid receptor (hMOR), human delta opioid receptor (hDOR), D2 dopamine receptor (DRD2), D4 dopamine receptor (DRD4), serotonin receptor 1B (5HT1B), and serotonin transporter linked polymorphism region (5HTTLPR) which may cause an imbalanced release of neurotransmitters in the brain, eventually resulting in the development of impulsive and addictive behaviours like drug abuse, smoking, gambling and drinking.

In the present study, a structured-interview using a questionnaire together with two personality trait assessments (Chinese version) were obtained for each club drug user to ascertain the growing trend of club drug use in the Hong Kong Chinese population as well as to determine the association of sensation seeking and harm avoidance personality traits with club drug use respectively. The questionnaire examined the demographics, pattern of drug use, experiences of first time drug use, current drug use, effects of drug use, potential dependence/withdrawal and knowledge about drugs of

abuse. The two personality trait assessments were the sensation seeking scale form V (SSS-V; M. Zuckerman, 1994) which is composed of four subscales, namely, 'thrill and adventure seeking', 'experience seeking', 'disinhibition' and 'boredom susceptibility', each of which has 10 forced-choice items. The other personality trait scale, the behavioural inhibition system and behavioural activation system scale (BIS/BAS; Carver and White, 1994) which is also composed of four subscales namely 'BAS fun seeking', 'BAS drive', 'BAS reward responsiveness' and 'BIS' (anxiety trait).

In addition, DNA samples were obtained from the club drug users to investigate whether an association exists between club drug use and personality traits with certain gene variants including the COMT G1947A $Val^{108/158}$ Met polymorphism, MAO-A T941G polymorphism, hDOR T921C polymorphism, hMOR A118G polymorphism, DRD2*TaqI* A polymorphism, DRD4 VNTR 48 bp repeat and -C521T polymorphism, 5-HT1B G861C polymorphism, and the 5-HTTLPR 44 bp insertion/deletion polymorphism.

Results showed that ketamine (88.5%) followed by marijuana (83.6%), 'ecstasy' (79.8%) and 'ice' (30.1%) were the most commonly lifetime use drugs in Hong Kong. Ketamine, marijuana, and 'ecstasy' were usually used in combination, with the occasional use of marijuana together with other stimulants or hallucinogenic drugs. Near to 90% and 60% of the club drug users have ever tried drugs during rave parties or discos in Hong Kong and in Shenzhen respectively (across the border).

Club drug users showed a significantly higher score in 'boredom susceptibility'

($p<0.0001$), 'disinhibition' ($p<0.0001$), 'experience seeking' ($p<0.0001$) and 'thrill and adventure seeking' ($p<0.001$) in the sensation seeking SSS-V scale; and the 'BAS fun seeking' ($p<0.0001$) and 'BAS drive' ($p<0.0001$) of the BIS/BAS scales. A significantly lower score in 'BIS' of the BIS/BAS scale was also shown in the club drug users when compared to the controls. There is a significant difference between male and female controls in 'boredom susceptibility' and 'disinhibition' in which males have higher scores. Females however have a higher BIS (harm avoidance) score. A similar trend was also observed between male and female club drug users with the addition of 'thrill and adventure seeking. The higher score for the 'thrill and adventure seeking' subscale in club drug users was merely contributed by male users. In general, male club drug users scored higher for 'boredom susceptibility', 'disinhibition', and lower for 'BIS' than female club drug users. The higher scores observed between the controls and the club drug users may be attributable to their chronic drug use.

Statistical significance was found between the club drug users and controls in the COMT $Val^{108/158}$Met polymorphism in both the genotype ($p=0.003$) and the allelic frequency with higher G allele ($p=0.013$). For the MAO-A T941G polymorphism, a significant difference ($p=0.012$) in allelic frequency with higher T alelle in the female subjects was found. For the hDOR T921C polymorphism, significant differences were found in both the genotype ($p=0.027$) and allelic frequency with higher C allele ($p=0.004$). Genotype frequencies of the DRD2*TaqI* A polymorphism ($p=0.011$) and the 5-HT1B G861C polymorphism ($p=0.015$) were also found to be significantly different between subjects and controls. However, no statistical significance was found in the polymorphisms of the hMOR, DRD4, and 5-HTTLPR genes.

When the interaction between personality traits and gene variants were investigated, it has been shown that club drug users with two copies of the G allele of COMT gene scored significantly lower than individuals with at least one of the A allele on the subscale 'boredom susceptibility' (p=0.02) and 'BAS drive' (p=0.01) while the control individual with at least one of the A allele of COMT gene scored significantly lower than individuals with two copies of the G allele on the subscale 'BAS fun seeking' (p=0.02). In the MAO-A T941G polymorphism in female club drug users, individuals with two copies of the T allele scored significantly lower than individuals with at least one of the G allele on 'BIS' (p=0.008) and individuals carried homozygous G allele scored significantly higher than individuals with at least one of the T allele on BAS reward responsiveness (p=0.024). In the hDOR T921C polymorphism, the control individuals with two copies of T allele scored significantly higher than individuals with at least one of the C allele on the subscales 'BAS reward responsiveness' (p=0.02) and 'BAS fun seeking' (p=0.004). In the *TaqI* A polymorphism of the DRD2 gene, club drug users with two copies of A1 allele scored significantly lower than individuals with at least one of the A2 allele on the subscales 'BIS' (p=0.01|). However, no interaction between personality traits and the G861C polymorphism in the 5-HT1B gene was found.

A significantly higher G allele frequency of COMT $Val^{108/158}$Met polymorphism and a higher C allele frequency of the hDOR T921C polymorphism were found in club drug users, and both these allelic variants were also associated with 'BAS fun seeking' in controls, it suggests that individuals who carry at least one of the G allele of COMT $Val^{108/158}$Met polymorphism or at least one of the C allele of the hDOR T921C polymorphism may be more susceptible to club drug use because of having high level

of 'BAS fun seeking' personality trait. However, the different association of personality traits and the same gene variant found between club drug users and controls might be the effect of chronic club drug use. A longitudinal study involving re-testing this same group of club drug users will further elucidate this. Moreover, it seems that multi-gene effects contribute to the effects of club drug use, in the present study, COMT, MAO-A, hDOR, DRD2, and 5HT1B gene variants were all found to be associated with club drug use of which both COMT and hDOR gene variants were also associated with 'BAS fun seeking' in the control group while both MAO-A and DRD2 gene variants were associated with 'BIS' in club drug users. It is therefore concluded from the present study that both gene variants and personality traits were associated with club drug use. In the future other gene variants in linkage disequilibrium with the current candidate genes studied should be examined to further establish a clearer role of the genetic contribution to personality traits and their link to substance abuse.

LIST OF ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic acid
5-HT	Serotonin
5-HT1B	Serotonin receptor 1B
5-HTTLPR	Serotonin transporter linked polymorphism region
ADHD	Attention deficit hyperactivity disorder
APD	Antisocial personality disorder
BAS	Behavioral activation system
BIS	Behavioral inhibition system
BS	Boredom susceptibility
χ^2	Chi-square test
cAMP	Cyclic AMP
CNS	Central nervous system
COMT	Catechol-*O*-methyltransferase
CPP	Conditioned place preference
CRDA	Central Registry of Drug Abuse
CREB	cAMP response element-binding protein
CSF	Cerebrospinal fluid
DA	Dopamine
DAT	Dopamine transporter
DI	Disinhibition
DRD2	D2 dopamine receptor
DRD4	D4 dopamine receptor
ES	Experience seeking
FDA	Food and Drug Administration
GABA	Gamma amino-butyric acid
GHB	Gamma-hydroxybutyrate
hDOR	Human delta opioid receptor
hMOR	Human mu-opioid receptor
IVE	Institute of Vocational Education
LSD	Lysergic acid diethylamide

MAO	Monoamine oxidase
MDA	N-demethylation
MDMA	3, 4-methylenedioxyamphetamine
METH	Methamphetamine
MPQ	Multidimensional personality questionnaire
NAc	Nucleus accumbens
NE	Norepinephrine
NEO-PI-R	NEO Personality Inverntory
NMDA	Glutamate N-methyl-D-aspartate
OPRD1	Opioid receptor delta 1
OPRM1	Mu-opioid receptor gene
OR	Odd ratio
PANSS	Positive and Negative Syndrome Scale
PCP	Phencyclidine hydrochloride
PET	Positron emission tomography
PFC	Prefrontal cortex
PKA	Protein kinase
PTSD	Posttraumatic stress disorder
SAD	Substance abuse disorder
SSS	Sensation Seeking Scale
TAS	Thrill and adventure seeking
TCI	Temperament Character Index
TH	Tyrosine hyroxylase
THC	Delta-9-tetrahydrocannabinol
TPQ	Tridimensional Personality Questionnaire
TS	Tourette syndrome
VNTR	Variable number of tandem repeats
VTA	Ventral tegmental area

實驗摘要

研究目的：近年香港青少年「狂野」派對中濫用精神科藥物情況嚴重，而在「狂野」派對中常見的藥物包括氯胺酮（俗稱「K仔」）、三甲氧安非他明（俗稱「Fing頭丸」）、甲基安非他明（俗稱「冰」）、大麻等。研究指出，擁有刺激追求或避免傷害人格特質的人較容易濫用藥物，而濫用藥物等行為卻被指與遺傳基因有關，所以相關的基因，包括多巴胺能的系統、血清素系統及阿片系統，可能與人格特質及濫用藥物有關。

方法：366名濫用精神興奮劑者會在訪問員接見下完成一份問卷（包括：個人基本資料，濫用藥物趨勢，第一次濫用藥物之經驗，服用藥物的原因，癥狀及潛在耐忍性或依賴性，對濫用藥物之認識及心理狀況）及兩份測量性格問卷（一份是由 Carver 與 White(1994)的行為抑制與行為激發系統敏感度測量表(BIS/BAS Scale)，此量表包含四個因素，分別為「行為抑制」(BIS)，而「行為激發」包含其餘三個因素，「酬賞反應」(Reward Responsiveness)，「趨力」(Drive)及「尋求樂趣」(Fun Seeking)；而另一份則是由 Zuckerman(1994)的刺激尋求量表五(SSS-V Scale)，此量表亦包含四個因素，名為「驚險及冒險活動尋求」(Thrill and Adventure Seeking)，「厭煩易感性」(Boredom Susceptibility)，「反抑制性」(Disinhibtion)及「經驗尋求」(Experience Seeking)，從而找出以上各種人格特質與濫用精神興奮劑的關係。另外，6毫升的血液會被抽取用作基因分析，在這實驗主要是研究以下幾個基因：兒茶酚-o-甲基轉移酶(COMT)基因 158/108 位密碼子、抑制單胺氧化酶A受體(MAO-A)基因 T941G 多態型性、μ阿片受體基因(MOR)的 A118G 多型性及δ阿片受體基因(DOR)的 T921C 多型性、多巴胺乙型(DRD2)基因的 Taq1A 型性、多巴胺第四型(DRD4)基因的-521C/T 及第三外顯子 48bp VNTR 多型性、5-羥色胺一乙型(5HT1B)基因的 G861C 多型性及 5-HTT 基因啟動區缺失／插入多態性，從而找出這群基因多型性會否與濫用精神興奮劑及人格特質有直接關係。

實驗結果：在366名濫用精神興奮劑青少年中，報稱濫用「K仔」佔 88.5%，其他較常濫用的藥物依次為大麻(83.6%)，「Fing頭丸」(79.8%)及「冰」(30.1%)。而「K仔」、大麻、「Fing頭丸」更是常被混合一起濫用的組合。分別接近90%的濫用精神奮劑青少年在香港或近60%在深圳曾經在「狂野」派對或的士高中濫藥。在性格量表中，濫用藥物者在「尋求樂趣」、「趨力」、「驚險及冒險活動尋求」、「厭煩易感性」、「反抑制性」及「經驗尋求」分數顯著比對照組高，但在「行為抑制」中顯著比對照組為低。在對照組中，女性在「行為抑制」中顯著比男性為高，但在「厭煩易感性」和「反抑制性」顯著為低，而相同的結果也在濫用藥物組找出，不過男性濫藥者「驚險及冒險活動尋求」顯著得分比女性濫藥者為高，所以，在「驚險及冒險活動尋求」中，濫葯組得顯著高分是直接受到男性濫藥者的分數反映。從基因多型分析結果中，COMT、MAO-A、DOR、DRD2及5HT1B的基因多型與濫用精神興奮劑有關。而在濫藥者中，COMT的基因多型與「厭煩易感性」和「趨力」有關，DRD2及MAO-A的基因多型與「行為抑制」有關，但在對照組中，COMT基因多型卻被發現與「尋求樂趣」有關，DOR的基因多型與「酬賞反應」及「尋求樂趣」有關。

總結：人格特質及遺傳基因與濫用精神興奮劑有關，多種基因多型性同時亦與濫用精神興奮劑及人格特質有密切關係。「尋求樂趣」主要是導致濫用精神興奮劑的人格特質，而長期濫用這些藥物亦會改變服用者的性格。所以，與濫用藥物相關基因有連鎖不平衡(linkage disequilibrium)關係之其他基因，是值得繼續研究，有助於深入了解基因及性格對濫用精神奮劑的關係。

CHAPTER ONE INTRODUCTION

1.1 Club drugs

Club drugs are chemical substances usually used recreationally to enhance social experiences. They first gained popularity in Europe in the 80s with the advent of the raves. Raves are all night dance parties with fast-paced and repetitive electronic music played by celebrated disc jockeys (DJs) and often accompanied by elaborate light displays. It embraced a party etiquette of "Peace", "Love", "Unity" and "Respect", which is captured in the rave logo "PLUR" (Weber, 1999). Raves centre around mobile sound system, musical blend of psychedelic hippiedom with combination of vigorous dancing and the liberal use of drugs such as 3, 4-methylenedioxyamphetamine (MDMA, 'ecstasy'), gamma-hydroxybutyrate (GHB), ketamine hydrochloride (ketamine), lysergic acid diethylamide (LSD), methamphetamine ('ice'; METH) and marijuana (Weir, 2000; 2004).

Over the years, the global trend of drug abuse has increased substantially and has become a problematic situation all over the world. According to the epidemiologic trends in drug abuse advance report generated by the Community Epidemiology Work Group (CEWG) in June 2002, 'ecstasy' is the most frequently used "club drug" in 20 states in the United States of America. The 2000 National Household Survey on Drug Abuse showed that 6.4 million American had ever tried 'ecstasy' at least once in their lifetime of which people in 12-17 increased 1.3 times to 615,000 people and those in 18-25 increased 3.18 times to 4,014,000 people. The US national school survey showed that high school and college-aged youngsters tended to use 'ecstasy' with an

increasing trend (Johnston et al., 2001; Strote et al., 2002). From the annual report on the state of drug problems in the European Union (EU) and Norway, 2002, cannabis continued to be the illegal substance most commonly used in all EU countries. Lifetime use of cannabis was reported to range from 10% (Finland) to 30% (the United Kingdom) of the whole adult population; amphetamines have been tried by 1% to 6% of the population, cocaine and 'ecstasy' have been tried each by about 0.5% to 4.5% of the population. Especially in the United Kingdom, 'ecstasy' and amphetamine are often taken in combination by drug users (Riley et al., 2001). From the statistics on drug use in Australia 2002 reported by the Australian Institute of Health and Welfare, it showed that recent illicit drug use was most prevalent among persons aged 20-29 years old followed by 14-19 years old. Around 35% people aged 20-29 years used at least one illicit drug and 30% used cannabis in the previous 12 months; about 10% used amphetamines and 'ecstasy'. Moreover, population-based studies showed that cannabis is consistently the most common illicit drug used (Lenton et al., 1997; Lynskey et al., 1999; Turner et al., 2003). In China, drugs including heroin, 'ecstasy', ketamine, and solvent use among teenage students had also been reported recently (Liu et al., 2001). Another study in China also showed that illicit drug use including heroin, ketamine and 'ecstasy' has continued to escalate across the six high-prevalence areas, namely, Guangzhou, Wenshan, Xi'an, Anshun, Lanzhou and Chongqing (Hao et al. 2002).

1.1.1 Pattern of "club drug" use in Hong Kong

Following the craze in western countries, attending rave parties have become a popular culture amongst young people in Hong Kong in recent years. "Club drug" use

in rave parties has also become popular amongst adolescents and young adults. In Hong Kong, of all the newly reported cases in 2002, 48%, 23%, 19.7% and 5.6% were known to abuse ketamine, 'ecstasy', marijuana and 'ice' respectively. For those under the age of 21, ketamine and 'ecstasy' are the most commonly used drugs. Their use increased dramatically from 1.1% in 1999 to 73.0% in 2002 and from 19.1% in 1999 to 35.8% in 2002 respectively (51st Central Registry of Drug Abuse, Security Bureau, Hong Kong SAR).

In line with the rapid economic growth in many cities and provinces in mainland China and the close connection between mainland China and Hong Kong, cross-border "club drug" use has become a new pattern among the young drug users in Hong Kong. Therefore, crossing the border to the border-town, Shenzhen, has become the main stream of cross-border drugs use among Hong Kong "club drug" users nowadays. This link of drug use pattern between Hong Kong and mainland China may probably be due to Hong Kong and Shenzhen having a common drug source.

International drug smuggling was mainly through the "China Channels" (places where there is a high prevalence of illicit drug use) from the "Golden Triangle" (where Thai, Burmese and Lao borders meet). The 'Golden Triangle' is notorious as a major production area of opium; it has now expanded its output to include large supplies of methamphetamine and manufacture of illegal stimulants including 'ecstasy' and cocaine. There is no physical barrier for Wenshan in Yunnan Province to the "Golden Triangle". The main route of drug smuggling starts from the "Golden Triangle" to Yunnan Province, then to Sichuan, Guizhou, Gansu, Shanxi Provinces, then from

these provinces to Guangzhou city, near to Hong Kong. Hong Kong or Guangzhou was the first place in China which is opened to the world and become one of the main drug trafficking centres (Hao et al. 2002). Therefore, "club drugs" in Hong Kong are mainly smuggled from mainland China. From the Security Bureau statistics in Hong Kong, it showed that "club drugs" smuggling has an increasing trend from 1999 to 2002, cannabis increased from 19.3kg in 1999 to 382.59kg in 2002, 'ecstasy' from 15,635 tabs in 1999 to 29,174 tabs in 2002, 'ice' from 21.92kg in 1999 to 26.01kg in 2002 but with a decrease in ketamine (from 45.09 kg in 2001 to 29.52 kg in 2002).

1.1.2. Popular "club drugs" used in Hong Kong and their effects.

1.1.2.1. MDMA ('ecstasy')

MDMA was first synthesized in Germany in 1914 and used as an appetite suppressant (Nichols et al., 1989; Green et al., 1995) but never produced commercially. MDMA had been used as an adjunct to psychotherapy in the late 70s and become popular at raves in the 80s. It has been a Schedule I drug since 1985 in the United States.

Street names for MDMA include 'ecstasy', X, ADAM, XTC, roll, M, lover's speed. It is available as a tablet, a capsule and a powder (Bethesda et al, 2000). The tablets usually contain 50 to 150 mg of MDMA (Graeme et al, 2000) and are formulated in different colours and imprinted with a popular icon like Nike swoosh, the Motorola logo and a butterfly. Other additives are usually found in the tablets including aspirin, caffeine, dextromethorphan, pseudoephedrine, ketamine and LSD (Baggott et al., 2000). In Hong Kong, 'ecstasy' usually appears in round tablets (30 to 40mg MDMA per tablet of various colours and logos with additives in different combinations like

MDMA, MDA, 'ice', ketamine, diazepam, paracetamol, caffeine and theophylline. (Hong Kong Government Laboratory, 2002 Annual Report).

MDMA is structurally similar to 'ice', hence it shares both stimulant and hallucinogenic properties (Schwartz and Miller, 1997a). Its effects on mood are mediated primarily by its activity on dopaminergic and serotonergic pathways (Milroy, 1999). MDMA increases the release of serotonin (5-HT), dopamine (DA), and norepinephrine from presynaptic neurons and prevents their metabolism by inhibiting monoamine oxidase (Nichols and Oberlender, 1989). It targets the serotonin transporter, stimulating 5-HT efflux and inhibits serotonin uptake (Rudnick et al., 1999). It results in an excessive amount of neurotransmitters available at the synapse; the excess 5-HT and DA are found to be responsible for the hallucinogenic effects (Nichols and Oberlender, 1989).

The effects of MDMA typically begin 30-60 minutes (min) after oral administration with the peak action at 60-90 min and typically last for eight hours (hrs) (Shulgin, 1986;Schwartz and Miller, 1997b). Metabolism of MDMA may be nonlinear particularly in higher-than-typical doses (de la Torre et al., 2000). MDMA is metabolized by N-demethylation to MDA, an active metabolite, but approximately two thirds of MDMA may also be excreted unchanged in the urine (Schwartz and Miller, 1997c), while MDMA is partially metabolized in the liver by the CYP2D6 isoenzyme of cytochrome P-450 (Tucker et al., 1994). Several genetic variants can affect an individual's ability to metabolize MDMA and may be the result of variable short or long term effects of MDMA (Solowij et al., 1992).

MDMA does appear to cause distortion and illusion, euphoria, feeling of intimacy, and increased energy; other effects are a distorted sense of time and diminished hunger and thirst (Schwartz and Miller, 1997d). It can elevate body temperature (Graeme, 2000) hence hyperthermia and dehydration may easily occur during hours of dancing. The combination of high environmental temperature, elevated body temperature, and increased muscular exertion associated with prolong dancing may lead to rhabdomyolysis and subsequent renal failure (Steele et al., 1994). Other side effects include trismus (tightening of jaw muscles) and bruxism (jaw-clenching), which prompt many MDMA users to suck pacifiers or lollipops to help relieve this sensation (Shannon, 2000). MDMA also caused rebound effects like generalized fatigue, muscle aches, difficulty in concentrating, anxiety, insomnia and depression lasting 1 to 2 days after ingestion (Parrott and Lasky, 1998).

Chronic MDMA use appears to have long-term neurotoxicity. Studies in animal models have shown that MDMA use decreases serotonin transporters resulting in relatively lower level of 5-HT in spinal fluid (Ricaurte et al., 1988) and causes the loss of 5-HT nerve endings (Bolla et al., 1998). In primates, this permanent neuronal damage was noticed seven years after exposure (Hatzidimitriou et al., 1999) while animals that showed signs of recovery often have abnormal patters of innervation (Fischer et al., 1995). Although the mechanism of neurotoxicity is not clear, it was hypothesized that MDMA cause 5-HT release from target neurons, causing the depletion of intraneuronal 5-HT. DA synthesis and release are in the mean time stimulated by MDMA, and excessive DA enters the nerve terminals once 5-HT is metabolized (Sprague et al., 1998). DA is then deaminated by monoamine oxidase, resulting in free radical formation and selective oxidative damage to the neurons

(Bolla et al., 1998). It was also found that there is impairment of long-term memory and learning in the offspring of rats exposed to MDMA, and that this consequence continued into adulthood (Broening et al., 2001). Studies in human have shown that MDMA induces serotonergic hypoactivity in users as evidenced by a significant reduction in the concentration of 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid and a decrease in striatal (caudate, putamen, nucleus accumbens) levels of 5-HT as measured by positron emission tomography (PET) (Kish et al., 2000). The amount of cortical 5-HT transporter and 5-HT uptake sites was also shown to be reduced (Obrocki et al., 1999; Semple et al., 1999). The patterns of glucose metabolism and blood flow in certain parts of the brain have been changed (McCann et al., 1998c) and the number of glial cells had increased (Chang et al., 1999). It clearly shows that MDMA can cause neuronal damage in humans even though the threshold dose for neurotoxicity has not been established. The serotonergic system modulates mood, impulse control, memory and some cognitive behaviour and it was shown that MDMA caused the decrease in 5-HT metabolites in the spinal fluid was correlated with a decrease in memory function (McCann et al., 1999) of users. A decrease in short-term memory (Zakzanis et al., 2001), visual (Steele et al., 1994), verbal (Krystal et al., 1992), working (Rodgers, 2000) and memory (Bolla et al., 1998) have also been reported.

1.1.2.2. Ketamine

Ketamine, a derivative of phencyclidine hydrochloride (PCP), was developed in the United States in 1962 and later patented by Parke-Davis in 1966 with trade names such as Ketaset and Ketajectm. Ketamine was promoted as a fast-acting general

anesthetic. It became widely used as an anesthetic during the Vietnam War (Siegel, 1978), and was approved by the Food and Drug Administration (FDA) for use among children and elderly in 1970. Currently, veterinarians use ketamine to induce sedation in animals for surgery, travel, and euthanasia with the maintenance of respiration and blood pressure (Curran and Morgan, 2000b). In pediatric patients, emergency reactions are less common and tend to be mild (Green and Johnson, 1990), while in adults, most adverse reactions can be controlled with the administration of benzodiazepines (Reich and Silvay, 1989). Its recreational use has become increasingly popular in the United States and Europe in the last decades and has been classified as a Schedule III controlled substance in 1999 in the United States.

Street names for ketamine include special K, K, kit-kat, super K and jet (Dillon et al., 2003). It is available as a powder that is usually ingested orally or nasally, or as a liquid that may be smoked after application to cigarettes or may be administered intravenously or intramuscularly (Weiner et al., 2000). In Hong Kong, it is sold as in powder form usually packaged in coloured paper packets (250mg per packet). The purity is ranged from 32% to 80% with additives including paracetamol, caffeine, aspirin and antipyrine (Hong Kong Government Laboratory, 2002 Annual Report).

Like PCP, ketamine interacts with the ion channel associated with the glutamate N-methyl-D-aspartate (NMDA) receptor (Krystal et al., 1994c) and binds non-competitively to the PCP receptor and inhibits glutamate activation of the channel. It affects opioid, DA (activates DA systems), 5-HT, noradrenaline (NE), nitric oxide, gamma amino-butyric acid (GABA, an inhibitory messenger), acetylcholine and endocrine system. It has also been found to inhibit the neuronal uptake of

norepinephrine, DA and 5-HT (Kohrs et al., 1998). A PET study showed that ketamine stimulates the release of DA from nerve terminals (Smith et al., 1998).

Ketamine is fast acting with the redistribution half-life of less than 5 min and last approximately 30-45 min (Jansen, 2000). At 10-25% of an anaesthetic dose, effects begin about 30 seconds after an intravenous injection, 2-4 min after an intramuscular injection and 10-20 min after taking the drug orally (Jansen, 2000c). It is metabolized by the hepatic cytochrome P-450 system to the metabolite norketamine and excreted in urine (Bolze et al., 1998). With long-term use of ketamine, both tolerance and hepatic enzyme induction have been shown (Lim, 2003).

Dissociative effects of ketamine have been shown at lower doses while amnesia occurred at higher doses (Jansen, 2000b). Its dissociative effects include hallucinations, perceived out-of-body experiences, slowed time perception and changes in the sensibility of environmental stimuli (Krystal et al., 1994b). Ketamine induces the cardiovascular toxicity from reflex sympathetic activation by inhibiting the reuptake of catecholamines resulting in mild to moderate increase in heart rate, blood pressure and overall cardiac output (Reich and Silvay, 1989). Other common effects like anxiety, hallucinations, nystagmus and vomiting are also developed (Krystal et al., 1994a). Some users may describe visit to the "K-hole", a place referring to where users are under the influence of ketamine (Tori, 1996). It can sometimes generate the features of a "near to death experience", including buzzing/whistling sounds at the beginning, travelling through a dark tunnel into light at a high speed, communication with God, intense visions and out-of-body experiences (Jansen, 2000a).

A report showing that ketamine could cause toxic changes in the rat brain (Olney et al., 1991b) and a high single dose or daily dose for 4 days can destroy neurons in the posterior cingulate and retrosplenial cortices and other corticolimbic brain regions (Olney et al., 1991a). In humans, ketamine has been shown to affect cognitive functions like, prose recall, speed of comprehension, letter fluency and digit cancellation indicating impaired episodic, semantic and working memory; with some of these impairments persisting even three days after drug use (Curran and Morgan, 2000a). Cases of chronic abuse leading to impaired memory or attention have also been reported (Jansen, 1990). One study illustrated ketamine-induced dependence, since it was shown knockout mice of NMDA receptor epsilon1 subunit gene markedly reduced the effect of ketamine, and eliminated the time-dependent sensitivity to ketamine (Sato et al., 2004).

1.1.2.3. Cannabis

Cannabis is the flowering tops and leaves of the plant Cannabis sativa, subspecies indica. It secretes a resin containing psychoactive compounds named cannabinoids which has the highest concentration in the flowering tops, followed by the leaves. The plant usually is cut, dried and incorporated into cigarettes with or without tobacco. Three types of plant preparations are used with the Indian names bhang, ganja and charas (Grinspoon and Bakalar, 1993). Bhang and ganja are referred as marijuana and charas is prepared from the resin itself that is 5 to 10 times stronger than marijuana. It has been used for both medicinal and non-medicinal purposes for thousands of years. The use of cannabis spread from China and the Middle East to Europe and then to

America in the middle of 19th century. It was made illegal in the United States in 1937 and has been continuously used as a recreational substance worldwide since that time.

The street names for marijuana include Pot, Reefer, Grass, Weed, Dope, Ganja, Mary Jane, or Sinsemilla. Most users smoke marijuana in hand-rolled cigarettes named joints, pipes or water pipes called bongs and marijuana cigars called blunts. The active chemical in marijuana is delta-9-tetrahydrocannabinol (THC), which is thought to cause the psychoactive effects of marijuana intoxication. Once smoked, the effects of marijuana begin immediately and last from 1 to 3 hrs and it deposits several times more THC into the blood than if ingested orally (Adam and Martin, 1996). THC attaches to cannabinoid receptors on nerve cells in several brain regions, affecting the way those cell functions by inhibiting the activity of adenylate cyclase and hence the formation of cAMP, a second messenger of the cells. THC also activates the reward system by stimulating brain cells to release the chemical DA and disrupts coordination and balance by binding to receptors in the cerebellum and basal ganglia which regulate balance, posture, reaction time and coordination of movement (Adams and Martin, 1996).

The primary effects of using marijuana recreationally are pleasant sensations, changes in perception, and time appears to pass very slowly. Since effects vary depending on dosage, users may become suddenly very hungry and thirsty; mouth feels dry, trembling hands and growing cold. Sleepiness or depression may also develop after the euphoria. Long-term effects include lung and throat problems such as coughing and increased frequency of throat and lung infections.

Cannabinoids have been shown to increase the release of NE, DA and 5-HT, especially the release of DA from rat corpus striatum, nucleus accumbens and medial prefrontal cortex. GABA turnover is enhanced by cannabinoids (Pertwee, 1992}. Some animal studies indicate the chronic cannabis use producing morphological changes in synapses as well as hippocampal neuronal loss (Eldridge and Landfield, 1990). Tolerance develops resulting from the pharmacological effects of cannabinoids in a variety of animal species, including pigeons, rodents, dogs, monkeys and rabbits (Rodriguez de Fonseca et al, 1994; Romero et al., 1995). Jones et al. (1983) showed that tolerance to the subjective effects of THC developed after oral administration of 10 mg for several days and greater tolerance developed with increased amount of the drug (Jones, 1983). They also showed that if the dose of THC is small and infrequent, little behavioral tolerance develops.

1.1.2.4. Methamphetamine ('Ice')

'Ice' is a potent, dependence-forming, central nervous system (CNS) stimulant. Street names for 'ice' include speed, chalk, ice, crystal, or glass. It is a white, odourless, crystalline powder that easily dissolves in water or alcohol. It can be smoked, snorted, orally ingested, or injected. The crystals may also be round shaped and made into tablets, and sold under the guise of 'ecstasy'. 'Ice' is usually carried in plastic bags or aluminium foil packets; a 1g plastic bag 'ice' is named a 'stroke'. It is usually taken by heating the crystal on a strip of foil and sucking the smoke through a liquid-filled hookah device to cool it off before it enters the body (Leung, 2002). Since it has a high potential for abuse, 'ice' is a Schedule II stimulant.

'Ice' is a powerful stimulant, even in a small dose can increase physical activity and wakefulness but decrease appetite. High dose can increase body temperature and respiration resulting in hyperthermia. Users can experience an intense rush and extreme pleasure, which lasts only a few minutes. The onset duration is 3 to 5 min by snorting and 15 to 20 min by oral ingestion. The mechanism of action is mainly due to the excessive release of DA (Jacobs and Fehr, 1987).

Long-term use of 'ice' can result in memory loss, aggression, violence, psychotic behaviour, and potential cardiac and neurological damage. Early findings revealed that high and repeated doses of 'ice' in monkey and rat caused long-lasting depletion of DA and decreased the activity of tyrosine hydroxylase in brain (Seiden et al., 1976). Other animal studies also showed that the same dose of 'ice' reduced DA levels and TH activity in brain with prolonged reductions of 5-HT levels. It was suggested that 'ice' produces neurotoxicity to these systems associated with motor and cognitive systems (Robinson and Becker, 1986; Volkow et al., 2001).

1.2 Neurobiology of drug addiction

1.2.1 Introduction to reward pathways and animal models of addiction

Addiction is the result of the adaptation in specific brain neurons caused by repeated exposure to drugs of abuse. Addiction can be characterized by three major elements: (1) seeking and taking drugs compulsively; (2) loss self control in limiting dose; (3) emergence of a negative emotional state when drug access is inhibited (Koob and

Nestler, 1997). Drug addiction can be regarded as the disease of the brain reward system. The reward system is composed of pleasure and punishment systems that help in the seeking and craving of life's essentials such as food, water, and social contact. Such natural rewards allow the subject to feel pleasure and these pleasurable feelings can reinforce the same behaviour to be repeated. Electrical self-stimulation, an animal model study, are widely used to determine the brain regions that appear to mediate reward, animals with electrode implanted in these areas in such a way that electrical impulses produce a pleasurable sensation will repeatedly do any required task to receive further electrical stimulation (Koob et al., 1989). Another animal model of addiction, namely the operant intravenous drug self-administration (IVSA) model, in which drugs of abuse are readily self-administered intravenously by animals, and in general, drugs that are self-administered correspond to those having high abuse potential (Niesink et al., 1999). These studies have shown that particular limbic structures are associated with the reward pathway including the dopaminergic neurons of the ventral tegmental area (VTA) that project to the nucleus accumbens (NAc; a nucleus in the limbic system that controls emotion and behaviour, specifically perception, motivation, gratification and memory), amygdala helps to assess whether an experience is pleasurable or aversive and helps to forge connections between an experience an other cues, prefrontal cortex where and other forebrain structures (Koob et al, 1989).

The conditioned place preference (CPP) animal study is used to show the learning association between environment stimuli and drug effect (Green and Brown, 2002). This experimental procedure provides an animal model of the subjective effects of drugs. A drug is injected and the subject is placed in a test chamber with distinctive

environmental cues. This procedure is repeated for several days. During these conditioning trials the animal develops an association between the subjective state produced by the drug (e.g., reward comparable to mood elevation and euphoria in humans) and the environmental cues present during the drug state. When the subject is tested in an apparatus that contains the drug-related environmental cues in one compartment and neutral cues in another, it voluntarily moves toward the compartment containing the drug-related cues. It was already suggested that brain regions other than that in reward take part in providing input to the mesocorticolimbic pathway (see 1.2.2.) concerning emotional and motivational variables. The association in "learning" of the drug and drug-related cues combined with complex interaction of psychological effect of drugs on brain areas associated with emotion and motivation may result in drug dependence. These learning processes mainly depend on the same motivational and emotional system in the brain once it was activated by the psychoactive substance (Schultz, 1998; Kelley and Mittleman, 1999; Schultz et al., 2000).

1.2.2 Mesocorticolimbic dopamine pathway

An essential part of this drug reward pathway appears to be the mesocorticolimbic pathway in Figure i (the proposed sites of action of the various drugs of abuse also shown in the pathway). This pathway is made up of axons of neuronal cell bodies in the VTA projecting to the NAc.

It also connects the VTA to prefrontal cortex. Ventral tegmental neurons release DA to activate the cells in NAc and regulate the activity in prefrontal cortex. Thereby,

stimulation of DA transmission in the mesocorticolimbic system is crucial mechanism of drug addiction.

The importance of DA in the reward pathway can be shown by several studies, for example, synaptic dopaminergic transmission in the NAc increases during natural rewarding action, like feeding or drinking as well as the administration of substances inducing addiction (Di Chiara and Imperato, 1988; Pontieri et al., 1995). The NAc is the key region that mediates the rewarding effects of drugs such as amphetamine and cocaine, which act directly by increasing the levels of DA at this site. Rats will self-administer tiny injections of amphetamine to the NAc by pressing a lever. More of the amphetamine was injected when their DA receptors were blocked pharmacologically, driving them to self-regulate the level of DA activity (Hoebel, et al. 1983). Many "knockout" studies have shown that mice lacking the DA D2 (DRD2) receptor consume less alcohol than intact controls (Phillips et al., 1998).

Thus, in the absence of DA receptors, the reinforcing properties of amphetamine disappeared. It was shown that when the neurotoxin 6-hydroxydopamine (a neurotransmitter analogue that depletes noradrenergic stores in nerve endings and induces a reduction of dopamine levels in the brain) was administered to cause a massive depletion of DA, rats no longer self-administer amphetamine and cocaine (Wise, 1987). Animal studies showed that similar reinforcing effects were also demonstrated when rats self-administer morphine to the NAc (Bozarth and Wise, 1984). It was further shown that there is a decrease in DA in the NAc during withdrawal from several drugs like morphine and amphetamine (Rossetti et al., 1992). Instead of looking at the tonic (minute-to-minute) level of extracellular DA in drug

addiction, one study illustrated the role of phasic (subsecond) DA signalling, DA was measured every 100 ms in the NAc using electrochemical technology. Rapid changes in extracellular DA concentration were observed at key aspects of drug-taking behaviour in rats. Before lever presses for cocaine, there was an increase in DA that coincided with the initiation of drug-seeking behaviours. Notably, these behaviours could be reproduced by electrically evoking DA release on this timescale. After lever presses, there were further increases in DA concentration at the concurrent presentation of cocaine-related cues. These cues alone also elicited similar, rapid DA signalling, but only in animals where they had previously been paired to cocaine delivery. These findings reveal an unprecedented role for DA in the regulation of drug taking in real time (Phillips et al., 2003). With the change in synaptic DA concentration, DA receptors undoubtedly play a crucial role in rewarding behaviours. Almost all psychoactive substances with reinforcing properties activate mesolimbic DA, either directly or indirectly. Therefore, it showed the crucial role of DA in the development of dependence for all classes of drugs because of its important role in response to reinforcement learning.

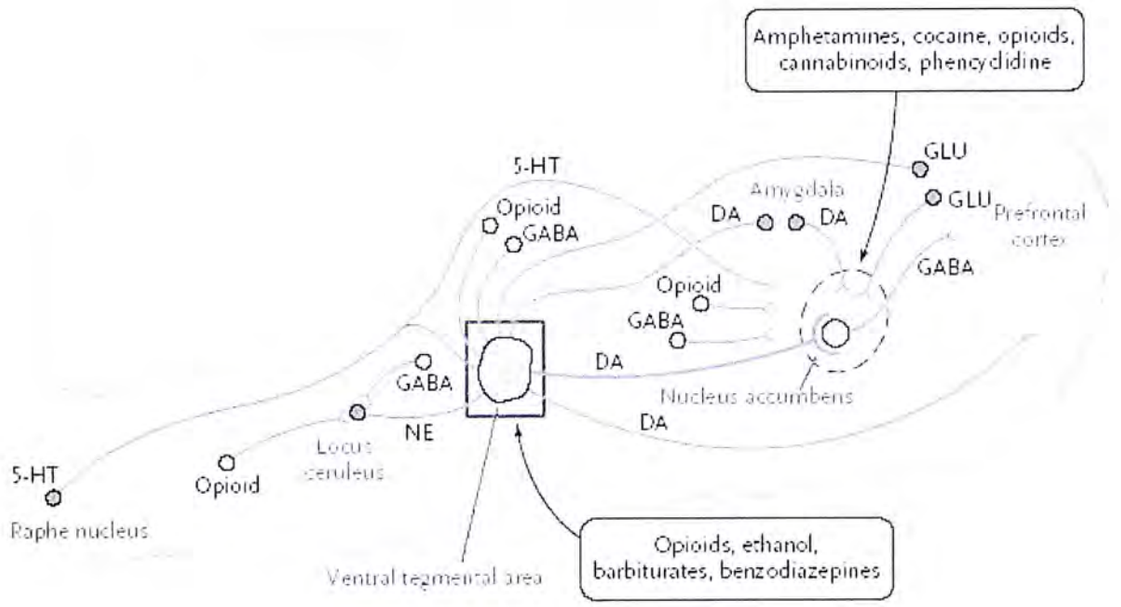


Fig. i The mesocorticolimbic pathway
As shown in the rat brain, mesocorticolimbic dopamine (DA) systems originating in the ventral tegmental area (VTA) include projections from cell bodies of the VTA to the nucleus accumbens (NAc), amygdala, and prefrontal cortex (PFC); glutamatergic (GLU) projections from the PFC to the NAc to and the VTA; and projections from the γ-aminobutyric acid (GABA) neurons of the NAc to PFC. Opioid interneurons modulate the GABA-inhibitory action on the VTA and influence the firing of norepinephrine (NE) neurons in the locus ceruleus. Serotonergic (5-HT) projections from the raphe nucleus extend to the VTA and NAc. **Source: Cami and Farre, 2003**

1.2.3 Behavioural processes of addiction

Dependence-producing substances share the ability to produce persistent changes in brain regions that involved in the process of incentive, motivation and reward, and such changes make these regions hypersensitive (sensitized), resulting eventually in the complex behaviours like dependence, tolerance, sensitization and craving characterized in addiction. This main motivation circuitry takes part in the cortical-striatal-thalamic-cortical loops: a loop of the projection of axon from prefrontal cortex to NAc, ventral globus pallidus, then to the thalamus and finally back to the cortex **(Fig. ii)** (Chambers et al., 2003). NAc allows various motivations to

take place by changing the neuroplasticity of different neurotransmitters on their own path in this motivation circuitry.

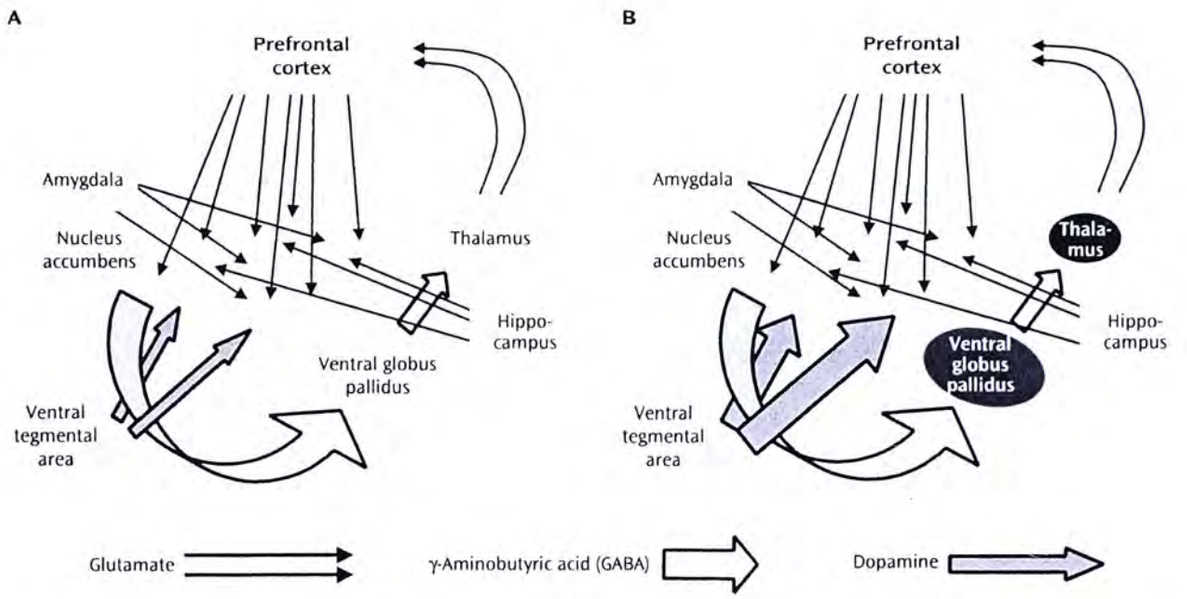


Fig.ii Part A shows the Cortical-striatal-thalamic-cortical loops within primary motivation circuitry involved in the representation of motivated drives and the neurocomputational events of motivational decision making and behavioural instigation.
Part B shows that DA discharge in the NAc (thickened arrows) is implicated in the identification of environmental novelty, the actions of addictive drugs, and the gating of motivated drives into behavioural actions by changing responses of NAc neurons to cortical and limbic glutamatergic afferents. These events are proposed to lead to relative extremes in firing patterns among NAc neuronal ensembles, depicted as increases in local peak amplitudes that code for behaviourally activating events in downstream motor systems. These events may also facilitate mechanisms of neuroplasticity among NAc neurons and their afferents, determining the future repertoire of motivational drive representations and/or thresholds for behavioural instigation.
Source: Chambers et al., 2003

Exposure of the brain to psychoactive substances can activate the mesolimbic DA system with the primary role in guiding motivated behaviour. Repeat exposure of drug can lead to strong association mediated by DA and the brain becomes more sensitive or "sensitized" to the motivational and rewarding effects of psychoactive substances. Thereby, drug induced adaptations that enhance drug responsiveness with repeated drug exposure, more compulsive patterns of drug seeking and taking behaviour are found. This sensitization has been shown for various drugs including amphetamine, cocaine, morphine, PCP, 'ecstasy', nicotine and ethanol (Robinson and Berridge, 1993). Increasing the presynaptic DA release and changing the postsynaptic receptor

sensitivity in the mesolimbic DA system result in sensitization. The idea of the transition from substance use to substance dependence may be closely related to the phenomenon of sensitization (Deroche et al., 1999).

Tolerance refers to drug induced adaptations that lead to diminishing effects of a constant drug dose (Chao et al., 2002c). Some drug effects are increased upon repeated drug use. Rapid tolerance develops to the lethal effects of amphetamine and cocaine (Hoffman and Goldfrank, 1990). Tolerance can be developed by the changes in receptor number or sensitivity together with the presence of sensitization and an increase with repeated drug administration (Berke and Hyman, 2000).

In short, possible relationships occur between neuronal plasticity of the related pathways in the brain as well as in the behavioural changes that result in addiction. Drug related behaviours may be contributed by several aspects of neural plasticity that are associated with repeated drug use in addicts: increasing the incentive motivational effects of drugs and enhancing craving for drugs are mediated by the change in neurotransmission between VTA and NAc in sensitization. Further increases in the tendency of drug related cues to drive drug-seeking behaviours by the enhancement from stimulus reward learning occur through the changes in neurotransmission between basolateral amygdala and NAc. Impairment of inhibitory control in governing reward-seeking behaviour may be caused by the changes in neurotransmission between PFC and NAc. Substance dependence can eventually induce the long lasting and nearly permanent behavioural changes, thereby the neuronal changes take a crucial role in this induction by changing (1) the synaptic plasticity in signalling; (2) the synaptic structure, and (3) the number of

neurotransmitter receptors.

1.2.4 Other neurotransmitter systems in addiction

Other neuronal pathways including neurotransmitters like 5-HT and glutamate also take part in regulating the activity of the mesolimbocortical DA pathway and mediating the rewarding properties of drugs of abuse.

Studies showed that the dopamine transporter (DAT) also took part in reward. Excess DA in synaptic gap is recycled by the transporter, which facilitates re-uptake by the presynaptic neuron. However, cocaine, amphetamine and methylphenidate block the re-uptake of DA by binding to the DAT and result in the increase of DA in the synapse, therefore the reinforcing effects of cocaine and methylphenidate have been linked to their ability to block DAT (Volkow et al., 1998). PET imaging using [^{11}C]cocaine as a DAT ligand showed that DAT occupancies were significantly correlated with the "high" of the effect of cocaine use (Volkow et al., 1999). However, in some other human brain-imaging studies, many subjective responses to cocaine do not correlate with its action at the DAT level implying that other system besides the dopaminergic system may also be involved in reward.

DA is one of the three catecholamines which are removed by reuptake into terminals, or into surrounding glial cells by Na^+-dependent transporters and enzymes responsible for its catabolism may also play a role in reward. The two major enzymes involved in the catabolism of DA are monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT), both of which are present within the

dopaminergic nerve terminals and are the targets of numerous psychoactive substances. Both COMT and MAO are the enzymes that catalyse the catecholamine neurotransmitters. Glutamatergic synapses located in the motivation circuitry play a role in memory and motivation by its projection to the NAc (Fig. 2) (Chambers et al., 2003). The glutamate receptors, which are coupled to sodium channels or the potassium channels through a G protein, therefore are important for learning and play an essential role in the hippocampus. Hallucinogens, such as PCP act at the NMDA subtype of glutamate receptor. It is thought that glutamate pathways play a very important role in modulating neural responses to many other psychoactive substances by the direct effect of NAc DA discharge pattern. By this interaction between glutamatergic and dopaminergic system, DA can reinforce an association between a particular set of stimuli and a particular behaviour response. The associative learning mechanism is developed in association with addictive drugs. It could similarly promote a tendency to respond to drug related cues and context (Berke and Hyman, 2000). Many studies showed that glutamatergic transmission takes part in drug addiction.. Chronic co-administration of glutamate receptor antagonists, like NMDA receptor antagonists, can attenuate the development of tolerance to the analgesic effects of opiates and the development of locomotor sensitization to several drugs of abuse (Robinson and Berridge, 1993; Trujillo and Akil, 1994). NMDA antagonists including PCP and MK-801 have strong stimulant and reinforcing actions of their own and potentially activate the reinforcing action of drug of abuse (Carlezon et al, 1996).

5-HT neurotransmitter system has recently been highlighted since there is a mutual inhibitory interaction between the 5-HT and DA system. 5-HT suppresses intracranial

self-stimulation (ICSS) thresholds while serotonergic antagonists facilitate this behaviour (Niesink, 1999). Mice without the 5-HT1B serotonin receptor are more likely to self-administer cocaine (Rocha et al., 1998b). PET study showed that abstinent 'ecstasy' users had decreased global and regional brain 5-HT transporter (5-HTT) binding compared with never used 'ecstasy' controls. Decreases in 5-HTT binding positively correlated with the extent of previous MDMA use (McCann et al., 1998b). Anti-depressant drugs shared with cocaine a high affinity for the 5-HTT molecule. Commonalities and co-morbidity between many forms of drug dependence and depression strengthen this link (McCann et al., 1998a). 5HT projects from the midbrain raphae nuclei to motivational circuit including VTA, NAc, PFC, amygdala, and hippocampus (Chambers, et al., 2003). Serotonergic deficit like a decrease in serotonin synaptic activity as well as lower level of platelet monoamine oxidase B (MAO-B) activity was found to be associated with pathological gambling (Ibanez et al., 2003).

1.2.5 Molecular plasticity in addiction: signaling and gene expression

Chronic administration of drugs of abuse induces changes in transcription factors, which are nuclear proteins that bind to the regulatory regions of certain genes, thereby regulating their transcription. Drug-induced changes at the level of gene expression could explain the longevity of the behavioural abnormalities associated with addiction. Different level of adaptive response at molecular level can be resulted in differences in vulnerability to dependence to different drugs (Nestler and Aghajanian, 1997).

Chronic exposure to opiates, cocaine, and alcohol up-regulates the cAMP pathway in

the NAc (Terwilliger et al., 1991; Unterwald et al., 1993; Nestler and Aghajanian, 1997). Drugs inhibit the re-uptake of DA in the VTA neurons (Volkow et al., 1999), where DA is initially produced from the amino acid tyrosine. DA is shown acting at the two main families of DA receptors (D1 and D2). These receptors are coupled to guanine-nucleotide-binding proteins (Gs and Gi), components of the intracellular cyclic AMP (cAMP) system, which also includes adenylyl cyclase and cAMP –dependent protein kinase (PKA). Possible substrates for this kinase include ion channels and the nuclear transcription factors CREB (cAMP response element-binding protein), c-Fos and c-Jun.

CREB is a transcription factor, a protein that regulates the expression, or activity, of genes that code for proteins and these proteins are then used to carry out the functions of the cell and thus the overall behaviour of nerve cells. When drugs of abuse are administered, DA concentrations in the NAc rise, inducing DA-responsive cells to increase production of a small signalling molecule, cAMP, which in turn activates CREB. After CREB is activated, it binds to a specific set of genes, triggering production of the protein those genes encode. Hence, activation of CREB is downstream of the cAMP-signalling pathway, whose up-regulation has been extensively characterized as an adaptation to chronic exposure to drugs of abuse (Guitart et al., 1992). The up-regulation of CREB was also discovered in the culture of neuroblastoma and gliomacells (Sharma et al, 1977) and in neurons in response to repeated opiate administration (Sharma et al, 1977). Short-term administration of opioid activates the mu-opioid G protein Gα i/o-coupled receptor, which inhibited adenylyl cyclase, lowered cAMP level, decreases cAMP-dependent PKA activity, and reduces phosphorylation of the transcription factor, CREB. Activation of mu-opioid

receptors can also cause the phosphorylation of CREB and other transcription factors. Decrease in the number of opioid receptors had been related in some reports to the development of opioid tolerance. Continuous stimulation desensitizes opioid receptors, which become phosphorylated by G-protein-coupled receptor kinases, beta-arrestins then bind to the receptors, causing them to be internalized by the neuron (Nestler, 2001; Berrendero et al., 2002; Watts et al., 2002).

Chronic drug use also causes sustained activation of CREB. For example, the chronic use of cocaine or amphetamine associated with activation of D1-like dopamine receptors (D1 and D2 DA receptors) stimulates a cascade of events. This includes the activation of Gs proteins, an increase in the formation of intracellular cAMP which lead to phosphorylation of transcription factors including CREB and to the induction of immediate-early genes such as c-fos and c-jun, whose expression is induced within minutes of exposure to a stimulus (Nestler et al., 1995; Hyman et al., 1996).

Chronic activation of opioid receptors produces effects opposed to those of acute activation. It up-regulates cAMP signalling pathways by increasing the activity of adenylyl cyclases, cAMP-dependent protein kinase A, and tyrosine hydroxylase (TH). Among the gene involved are adenylyl cyclase type VIII and TH whose expression is up-regulated by chronic morphine administration via the CREB-dependent mechanism. As a result, the phosphorylation of CREB and ΔFosB, factors regulating gene transcription is increased by activation of the cAMP cascade causing the influx of sodium, these changes correlate with the manifestations of the withdrawal syndrome (Lane-Ladd et al., 1997; Chao et al., 2002b; Chao et al., 2002a).

Fos family proteins respond to drugs of abuse with a characteristic sharp up-regulation followed by a quick decline to basal levels within hours (Young et al, 1991) but the isoforms of ΔFosB are very stable and demonstrate in vivo half-life of several weeks (Chen et al., 1997). It was shown that the presence of ΔFosB protein persisted for weeks even after the drug is withdrawn (Andresson et al., 2003). ΔFosB therefore plays a significant long-term role in subsequent regulation of gene expression. Transgenic mice over expressing ΔFosB showed that the NAc and dorsal striatum exhibit increased responsiveness to the rewarding and locomotors activating effects in response to chronic exposure to several drugs of abuse, including cocaine, amphetamine, opiates, nicotine, ethanol and PCP (Nye et al, 1995; Nye et al, 1996; Ryabinin et al, 1998; Kelz and Nestler, 2000) and enhanced drug-seeking behaviours (Chen et al., 1997; Nestler, 2002). So, ΔFosB becomes a unique target of chronic exposure to drug of abuse, it is deemed the 'molecular switch' that gradually converts acute drug response into relatively stable adaptations that contribute to the long-term neural and behavioural plasticity that underlies addiction (Nestler, 2001). By affecting the expression of many other genes within the same neurons with the accumulation of ΔFosB, the synaptic transmission as well as the neuronal functions in brain can be continuously affected, the long-lasting changes in neuronal composition will be resulted.

1.3 Association of Personality Traits and Drug Abuse

Substance use, like smoking, drinking, marijuana, heroin and cocaine, had been found to be associated to personality disorders. O'Malley et al. (1990) showed that substance abusers have a higher rate of DSM Axis II personality disorders than those

in the general population. To date, a number of studies have shown that there is an association between personality disorders and substance-use disorders. Gelernter et al. (1997) showed that both personality disorders and mood/anxiety disorders are consistently found to be highly prevalent among substance abusers. It was estimated that the prevalence of mood/ anxiety disorder among substance abusers to be in the range of 15-30% (Gelernter et al., 1997) and the prevalence of personality disorder to be as high as 30%-75% (Verheul et al., 2001). More specifically, there is a higher prevalence of smokers amongst the psychiatric population and the early onset of smoking was shown to be linked to depression and alcohol use disorder (Hanna, 1999). Major depression among alcoholics, with a rate of 36%, was two to three times more prevalent than non-alcoholics (Robins and Regier, 1991). Furthermore, at least two thirds of alcohol-dependent individuals showed evidence of anxiety, sadness, and antisocial behaviours (Schuckit et al., 1997). Anxiety symptoms are also common with marijuana use. Patients of substance abuse disorder (SAD) with the majority involved in marijuana and stimulant use showed that 60% of them had mood disorder, 63% with anxiety disorder and 80% with schizophrenia spectrum disorder (Swadi et al. 2003). Brooner et al. (1997) used the DSM-III-R criteria for antisocial personality disorder (APD) showed that APD is the most frequent personality disorder among the opioid abusers, (15% in females and 35% in males) (Brooner et al., 1997). When cocaine and heroin abusers were compared, heroin abusers (45%) showed a higher proportion of APD than in cocaine abusers (25%) (Fieldman et al., 1995).

With the increase in comorbidity of substance dependence and mental illness, more studies emerged illustrating the personality traits of the substance abusers (Francis et al., 1997; Cooper et al., 2000; LoCastro et al., 2000; Sher et al., 2000; Soloff et al.,

2000). Biologically, the personality traits are based either having a shared neurobiological basis or having an interaction of effects at some levels. Three personality traits are biologically conceptualized by Eysenck namely extraversion, neuroticism, and psychoticism (Eysenck et al., 1985). Extraversion is characterized by being outgoing, talkative, high on positive affect, and in need of external stimulation, according to Eysenck's theory, there is an optimal level of cortical arousal, and performance deteriorates as one becomes more or less aroused to this optimal level. Neuroticism is characterized by high levels of negative affects including depression and anxiety. Neuroticism, according to Eysenck's theory, is based on activation thresholds in the sympathetic nervous system or visceral brain (Eysenck et al., 1985). This is the part of the brain that is responsible for the fight or flight response in the face of danger. Psychoticism is characterized by nonconformity, hostility, and impulsivity (Eysenck et al., 1985). The physiological basis revealed by Eysenck for psychoticism is testosterone, with higher levels of psychoticism associated with higher levels of testosterone. Eysenck's model is useful because it specifies some of the biological mechanisms believed to underlie personality traits which may be risk factors for substance use. Other biologically based models, such as Zuckerman (Zuckerman et al., 1984), Gray (Gray, 1987), Cloninger (Cloninger, 1987a) and NEO PI-R (Costa and McCrae, 1991) gave rise to their own models related to Eysenck's model.

Certain personality traits share the same biological determinants, namely, neurotransmitters, which takes part in the reward pathway of the brain. For example, higher DA and lower MAO levels in the cerebrospinal fluid (CSF) were found in high sensation seeking personalities (Zuckerman, 1985). Sensation seeking is similar to

dimensions related to impulsivity in Eysenck's and Cloninger's models (Zuckerman and Cloninger 1996; Zuckerman et al., 1993).

Sensation seeking has been related to various high-risk behaviours including sports, criminal activities, sexual behavior, smoking, heavy drinking, drug use and abuse (Zuckerman and Kuhlman, 2000). Zuckerman's Sensation Seeking Scale form V (SSS-V) is one of the commonly used scales to measure sensation seeking traits (Zuckerman, 1994). The SSS-V is composed of four subscales, each of which has 10 forced-choice items: (1) thrill and adventure seeking (TAS), with items expressing a desire to engage in sports or other activities involving speed or danger; (2) experience seeking (ES), which represents the seeking of experience through the mind and senses, travel and non-conforming lifestyle; (3) disinhibition (DI) which represents the desire for social and sexual disinhibition; and (4) boredom susceptibility (BS) representing an aversion to repetition, routine and restlessness when things are not changing. In the focus on the importance of sensation seeking as related to substance use, sometimes different names are used for sensation seeking, such as impulsivity (personality trait characterized by acting on impulse, non-planning, liveliness, and risk-taking) (Eysenck et al, 1985); behavioural approach (tendency to seek out pleasant stimuli) (Gray, 1987); novelty seeking (tendency to seek out-of-the-ordinary experiences) and reward dependence (approach motivation) (Cloninger, 1987a) .

Higher scores had been found on the subscales including impulsiveness, anxiety, sensation seeking, somatic complaints, obsessive-compulsive behaviour and psychoticism in 'ecstasy' users and even higher scores was found in heavier 'ecstasy' users (Daumann et al., 2004). A cross-sectional study showed that temperamental

characteristics were associated with an increased risk of substance use disorder in subjects with psychotic or mood disorder; subjects with higher SSS- subscales ES and DI scores were more likely to present with a lifetime history of alcohol abuse/dependence. Greater DI scores were also been shown to be associated with a lifetime use of cannabis (Liraud and Verdoux, 2000). Zuckerman (1999) has consistently found higher DI and ES scores related to drug use (Zuckerman, 1999). It had been shown that among young adult drug abusers, high sensation seekers tend to abuse all types of drugs, including marijuana, cocaine, amphetamines and barbiturates. The highest sensation seekers were polydrug abusers who abuse a variety of drug uses concurrently or over their lifetime (Ball et al, 1986).

Gray illustrated that there are two main behavioural systems, namely, the behavioural inhibition system (BIS) and behavioural activation system (BAS), in which they are mediated by the serotonergic system originating in the median raphe and the dopaminergic activity in the mesolimbic system respectively (Gray, 1994). BAS in Gray's model is closely related to extraversion in Eysenck's model, whereas BIS in Gray's model is closely related to neuroticism in Eysenck's model (Gray, 1987).

Craver and White (1994), based on the two main behavioural systems proposed by Gray (1987), had devised subscales to the BAS (brain system posited to underlay the tendency to seek out pleasant stimuli) and BIS (brain system posited to underlay the tendency to avoid unpleasant stimuli) systems. In this BIS/ BAS scales, BAS was divided into three subscales: reward responsiveness, drive and fun seeking. BIS was shown to reflect a greater tendency for anxiety and harm avoidance (capacity to learn and change behaviour as a result of punishment) (Carver and White, 1994). Using the

BIS/BAS system, it was shown that in individuals with a lifetime history of alcohol use, high BAS-fun seeking scores were found only among those without comorbid anxiety (Conrod et al., 2000; Johnson et al., 2003). Subjects with high BAS-drive scores experienced significantly stronger desires and intentions to drink alcohol and have negative reinforcement cravings (behaviors that terminate aversive effects are negatively reinforced, like the negative symptoms of withdrawal can be terminated by taking the drug again) during exposure to alcohol related cues than subjects with low BAS-drive scores (Franken, 2002).

As defined by Cloninger (1987b), type 1 alcoholics drink to reduce anxiety and distress while type 2 alcoholics drink for enjoyment and for the disinhibition produced by alcohol and are more likely to be engaged in fighting and other antisocial behaviours. Early onset of alcohol abuse and criminality has also been shown to be associated with higher novelty seeking behaviour (Howard et al., 1997). It is therefore evident that the association of personality traits and substance use is complex, that different personality traits may be associated with different patterns of drug use and that the different dimensions of personality traits can be characterized even within the one type of drug use.

1.4. Association between genetics and drug abuse

Drug use is a complex behaviour in which both genetic and environmental factors exert a major influence on an individual's vulnerability to drug abuse (Anokhin et al., 1999). Base on family, twin, adoption, case-control and linkage studies, there is a strong genetic factor that contributes to drug dependency; however, there is no single

gene that was shown to cause drug dependence. In other words, several distinct genes may combine to contribute to drug abuse susceptibility thus increasing the individual's risk of developing drug abuse.

1.4.1 Family, twin and adoption studies

Family, twin and adoption studies have revealed contribution of genetic factors to psychoactive substance use and dependence. It is supposed that if the proportion of monozygotic twins concordant for a given trait is greater than the proportion of dizygotic twins, it is likely that genetic factors are influencing the trait. If there is no significant difference in concordance rates between monozygotic and dizygotic twins, then the trait is likely to be influenced by environmental factors. The patterns of inheritance and relative risk of drug abuse can be examined from family studies by the inheritance of traits through a family. It was shown that there was an 8-fold increased risk of drug disorders among the first-degree relatives of 231 probands with drug disorders across a wide range of specific substances, including opioids, cocaine, cannabis, and alcohol (Merikangas et al., 1998). In a review of more than 17,500 reared-together monozygotic and dizygotic twins from 14 different studies, it was estimated that genetic, familial-environmental and individual-specific environmental risk factors accounted for 56%, 24% and 20% of the variance in regular tobacco use respectively and genetic factors contribute to near to 60% of the variance in smoking (Sullivan et al., 1999). A twin study on alcoholism in males showed a concordance rate of 63.4% for monozygotic and 43.8% for dizygotic twin pairs (Pickens et al., 1991). Another study on substance dependence with the twin pairs from the Vietnam Era Twin Registry showed that the genetic variance of heroin abuse was 38% but only

16% for marijuana and amphetamines abuse (Tsuang et al., 1998).

1.4.2 Transgenic and knock out animal models

Transgenic mice are genetically engineered by introducing a segment of DNA from a different organism into the germline of the mice that allow researchers to examine specific receptors that may mediate the addictive properties of drug abuse. For example, cultured cells (Hiremagalur et al., 1993) or transgenic mice with over-expressed TH, the rate-limiting enzyme controlling DA synthesis, was shown to be less sensitive to physiologic effects of nicotine (Nabeshima et al., 1994). In knock-out animals, the specific gene of interest will be replaced by an inactive or changed one, thereby a deficiency in protein expression corresponding to the gene can be observed and these transgenic mice is called knock-out mice. A knock-out mice lacking the DAT gene, the crucial binding site of cocaine, can retain the capability to self-administer cocaine intravenously (Rocha et al., 1998a). Psychostimulants and serotonin uptake inhibitors attenuated the hyperactivity of DAT knock-out mice (Powell et al., 2004). There was a suppression of rewarding behavior with morphine in DRD2 knock-out mice but these knockout mice displayed normal response when food was used as a reward (Maldonado et al., 1997).

1.4.3 Candidate genes for drug abuse

Receptor gene variant in the serotonergic, dopaminergic, GABAergic, opioid systems in the CNS which are shown to be associated with addictive behaviours like

alcoholism, cocaine, smoking, heroin dependence (Comings et al., 1997; Lappalainen et al., 1998; LaForge et al., 2000; Noble, 2000).

1.4.3.1 Dopamine receptor genes

The mesolimbic dopaminergic system has been shown to be involved in the reinforcing effects of many substance of abuse. Variants of the DA D2 receptor (DRD2) gene had been shown to be associated with alcohol, nicotine, cocaine and opioid dependence (Noble, 2000). The gene variant DRD2 TaqI A1 allele has been shown to be associated with reduced D2 DA receptor binding affinity (Noble et al, 1991). In alcoholic patients with the DRD2 A1 allele, a greater severity of alcohol dependence was shown (Connor et al., 2002).

A 48 base pair variable number of tandem repeats (VNTR) polymorphism in exon 3 of the DRD4 gene has been identified and is thought to play a role in novelty seeking behaviours, attention deficit hyperactivity disorder (ADHD), Tourette syndrome (TS), pathological gambling, and substance abuse (Comings et al., 1999). Further association between the L allele of DRD4 gene and alcohol dependence was also shown. Participants were asked to consume 3 alcoholic drinks or 3 control drinks and measures of craving were completed on them after each drink. It was shown that those with the L allele demonstrated significantly higher craving after consumption of alcohol as compared to those who drank the control beverage (Hutchison et al., 2002).

A polymorphism of the DAT has been identified which is associated with altered levels of the DAT protein in the brain (Heinz and Goldman, 2000), suggesting that the

polymorphism results in a functional difference. However, no association with substance dependence has been found.

1.4.3.2 Monoamine Oxidase (MAO) gene

MAO is encoded in X chromosome and the MAO-A locus is an attractive candidate for exploring genetic contribution to the variation in the risk for substance use disorders (SUD) (Vanyukov et al., 2003) because of its important role in the metabolism of neurotransmitters, including DA and 5-HT. The occurrence of a G at position 941 creates a restriction site for Fnu4H1 within the coding region of the MAO-A gene, one of the major enzymes responsible for the degradation of neurotransmitter such as 5-HT, NE and DA. The G allele was found in individuals with a higher MAO-A activity (Hotamisligil and Breakefield, 1991). Cigarette smokers have reduced brain levels of MAO-A (Fowler et al., 1996) and levels of MAO in peripheral tissues (Berlin et al., 1995). This may be due tobacco smoke contains MAO-A inhibitors which may contribute to the lower levels observed in smokers.

Significant associations of alcohol dependence with MAO-A alleles were found among the Han Chinese people, but not in Taiwanese (Hsu et al., 1996).

1.4.3.3 Catechol-*O*-methyltransferase (COMT) gene

COMT is the enzyme that catalyses the first step in one of the major degradative pathways of the catecholamine neurotransmitters, DA, epinephrine, and NE (Cooper et al., 1996). In particular, the frontal cortex is the area where COMT is responsible

for more than 60% of DA degradation instead of MAO (Karoum et al., 1994). A single base pair change (G→ A) in exon 4 of the COMT gene results in the creation of an Nla III site in the DNA and an amino acid change (Val, 108 COMT→ Met, 158 COMT). This single amino acid change from Val (H) to Met (L) results in a decrease of 3 to 4 fold COMT enzyme activity (Lotta et al., 1995; Lachman et al., 1996); the alleles are co-dominant, as heterozygous individuals have enzyme activity that is midway between homozygote individuals (Weinshilboum et al., 1999). In other words, individuals with L alleles presumably have relatively more baseline dopaminergic signalling at synapses while H alleles lead to increase DA catabolism resulting in relatively less DA in the prefrontal synapses. Thus, variations in COMT activity might affect prefrontal cortical activity.

A functional high COMT activity homozygous val allele has been shown to be associated with alcohol dependence and polysubstance use (Vandenbergh et al., 1997; Horowitz et al., 2000). Type I (with late-onset but without prominent antisocial behavior) alcoholism is more common among subjects with low activity COMT (met/met), compared to those with high activity val/val or heterozygotic (val/met) genotypes (Tiihnen et al., 1999). 896 middle-aged Finnish men with low COMT activity met/met genotype (30% of all subjects) were reported to have 27% higher weekly alcohol consumption when compared with the two other genotype groups, val/val and val/met (Kauhanen et al., 2000). However, the high COMT activity val/val genotype were reported in 18% of controls and 31% of the high lifetime substance user, hence the high activity COMT variant may have greater genetic vulnerability to drug abuse (Vandenbergh et al., 1997).

1.4.3.4 Serotonergic genes

5-HT is an important neurotransmitter that regulates various essential brain functions via many 5-HT receptor subtypes. There are seven main classes of 5-HT receptors among which the 5-HT1 receptor is divided into subclasses (A, D, E and F) on the basis of their pharmacologic and biochemical properties (Humphrey et al., 1993; Hoyer and Martin., 1996). In the CNS, 5-HT1B receptors are widely distributed in the basal ganglia, hippocampus and other regions of the cortex, and function as both 5-HT autoreceptors and heteroreceptors, mediating respectively, the release of serotonin and non-serotonin neurotransmitters (Barnes and Sharp, 1999; Moret and Briley, 2000). 5 HT1B knockout mice has been found to exhibit greater aggression and impulsivity (Saudou et al., 1994) and to have an increase in alcohol intake and cocaine consumption (Crabbe et al., 1994; Rocha et al., 1998b). It was therefore suggested that 5HT-1B is important in the development of alcoholism and other psychiatric disorders (Scearce-Levi et al., 1999). This was confirmed by a Finnish study in which Finnish antisocial alcoholics were found to have a higher frequency of the 861C allele in the 5-HT1B polymorphism G861C than Finnish controls (Lappalainen et al., 1998).

Variation in the serotonergic system is related to mood regulation, impulse control, appetite and aggression. Since 5-HTT is a functional protein that regulates the amount of 5-HT in a synaptic gap, the agents that bind to 5-HTT, for example, serotonin reuptake inhibiting antidepressants, cocaine and 'ecstasy' will modify serotonergic transmission. These agents elevate mood in depressed or normal subjects. Therefore, 5-HTT is one of the targets in the etiological study of psychiatric disorders including

mood and anxiety disorders

There is a 44 bp pair insertion/deletion polymorphism in the promoter region of the human serotonin transporter gene (5-HTTLPR) containing 'long' (L) and 'short' (S) alleles (Heils et al., 1995). The L allele was shown to have higher transcriptional activity in vitro and in lymphoblastoid cells (Heils et al., 1996; Lesch et al., 1996). The 5-HTT gene (SLC6A4) is a candidate gene for smoking and predisposition to alcohol dependence because of its association with psychological traits relevant to smoking and alcohol dependence. For example, a Japanese study found that 5-HTTLPR was associated with smoking (Ishikawa et al., 1999) and smokers with coronary heart disease (Arinami et al., 1999).

1.4.3.5 Opioid receptor genes

The human mu-opioid receptor (hMOR) plays a crucial role in the response to opioids and to both endogenous opioid peptides for the regulation of normal physiology of what and synthetic exogenous ligands (Kreek, 1996). Herz (1997) showed that reward behaviour was activated by β-endorphin, an endogenous opioid peptide that activates the μ-opioid receptor, the primary action site for heroin and endogenous β-endorphin. Mu-opioid agonists are important clinically in the management of pain and short acting opiates like heroin (Dole et al., 1996; Kreek, 1996). The hMOR A118G polymorphism has also been shown to be associated with heroin dependence in the Hong Kong Chinese population (Szeto et al., 2001) and associated with heroin and alcohol-dependence in the German population (Franke et al., 2001) and in the Indian population in Singapore (Tan et al., 2003).

The delta-opioid receptor (OPRD1) T921C polymorphism has been shown to be associated with heroin dependence in the German population (Mayer et al., 1997) and we have also an association in the Hong Kong Chinese population (unpublished data) although not in mainland Chinese population (Xu et al., 2002).

1.4.4 Linkage studies of drug abuse

Linkage studies use multiple affected families to examine traits that are inherited together. It is based on the fact that genes that are located close to one another will be more likely to be inherited together from one parent than two genes located further apart. When there is a greater chance of genes being inherited together, the genes are therefore said to be linked. Comings et al. (1997) showed that a linkage was found between loci on chromosome 5q and the locus for the DA D1 receptor that has been always associated with smoking. A significant sib pair linkage in southwestern American Indian to chromosome 6 was found to be associated with antisocial alcoholism (Lappalainen et al., 1998). The strongest linkage with loci for alcohol dependence are on chromosomes 1, 2 and 7 (Reich et al., 1998).

1.5 Genetic factor and personality trait

Majority of genetic researches on personality traits include self-report questionnaires administered to adolescents and adults are based on the definition that personality is the vigor, temper or persistence of behaviour or to the emotional expression that accompanies it like fearfulness, exuberance, aggressiveness or self-restraint (Loehlin,

1992). Numerous self-report questionnaires like revised NEO Personality Inventory (NEO-PI-R) (McCrae and Costa, 1997); Eysenck's Personality Inventory (Eysenck, 1952); Zuckerman's Sensation Seeking Scale (SSS) (Zuckerman, 1994); Cloninger's Tridimensional Personality Questionnaire (TPQ) (Cloninger, 1987a) and Temperament Character Index (TCI) (Cloninger et al., 1993) are in use and appear to reliably measure many of the same personality factors including novelty seeking (extraversion, conscientiousness, sensation seeking, psychoticism) and harm avoidance (neuroticism, anxiety-related traits).

Cloninger and colleagues have proposed a biosocial model of personality based on four temperaments (novelty seeking, harm avoidance, reward dependence and persistence) (Cloninger et al., 1993). Novelty seeking is referred to the tendency to respond actively to novel stimuli or cues or potential rewards and escape from punishing (behaviour activation). Harm avoidance corresponds to the tendency toward an inhibitory response to signals of aversive stimuli that lead to avoidance of punishment and non-reward (behaviour inhibition). Reward dependence is the tendency for a positive response to signals of reward to maintain or resist behavioural extinction. Persistence is the perseverance despite frustration and fatigue (behaviour maintenance). According to this model, three of the four temperaments were associated with a specific central neurotransmitter. Novelty seeking is associated to dopaminergic activity; harm avoidance to serotonergic activity and reward dependence to noradrenergic activity. Thereby, it was suggested that genes corresponding to the neurotransmitter balance in the brain could change the personality traits including impulsive, compulsive and even addictive behaviours (Comings, 1996).

1.5.1 Twins study

From the twin studies, it was shown that heritability of personality traits are in the 30-50% range. Ebstein showed that extraversion is one of the most highly heritable categories (~50%) followed by neuroticism (~40%) (Ebstein et al., 2000). The within sex genetic correlations between neuroticism and major depression was 0.68 in men and 0.49 in women (Fanous and Kendler, 2004). The following are the studies in which an association of personality and genetic polymorphisms were found. Monozygotic twins of combat-related posttraumatic stress disorder (PTSD) probands had significantly more mood disorder symptoms than monozygotic controls and dizygotic PTSD probands (Koenen et al., 2003). One twin study illustrated that the phenotypic correlations between the control scale of the multidimensional personality questionnaire (MPQ, Tellegen, 1982) and the four subscales of the sensation seeking scale (SSS) with 55% attributed by genetic variance and the rest was specifically attributed to the control scale of the MPQ (Hur et al., 1997).

1.5.2 Candidate gene studies

Associations between specific genetic polymorphisms and specific personality traits have been reported (Benjamin et al., 1996; Ebstein et al., 1996), especially polymorphisms corresponding to the neurotransmitter receptor genes were largely investigated.

1.5.3.1 Dopamine (DA)

Association of personality trait and the 48 base pair variable number of tandem repeats (VNTR) polymorphism in exon 3 of the DRD4 gene was reported. The long allele of the DRD4 was associated with higher novelty seeking (Noble et al., 1998; Kuhn et al., 1999; Strobel et al., 1999; Tomitaka et al., 1999) while some studies did not show the association (Vandenbergh et al., 1997; Bau et al., 1999). Recently, the DRD4 48bp VNTR polymorphism at exon 3 has been shown to be putatively associated with novelty seeking and other personality traits. DRD4 receptor knock-out mice exhibited reduced exploration of novel stimuli (Dulawa et al., 1999) whereas in humans, the DA D4.7 receptor variants have shown to be associated with novelty seeking behaviour (Benjamin et al., 1996; Ebstein et al., 1996). In a meta-analytical review of 20 studies (n = 3907), 13 reports suggested that the presence of longer alleles was associated with higher novelty seeking scores and seven showed the opposite results (Kluger et al., 2002).

However, the relevancy of DRD4 to personality trait cannot be overlooked. Another functional polymorphism was found in the 5' region of DRD4 gene, this –521C/T polymorphism is located in the region that regulates cell-type specific gene expression in which the –521T mutation showed significantly less transcriptional activity (Kamakura et al., 1997). An approximately 40% decrease in transcription efficiency if human retinoblastoma cells which transiently expressed –521T (Okuyama Y. et al., 2000). The –521C/T polymorphism was also shown to be associated with novelty seeking in the same study. Further, allele C had been shown to be associated with ADHD (Jonathan M. et al., 2003). Okuyama et al. (2001) showed a relevancy of the

promoter region of the DRD4 gene to novelty seeking; they showed that a promoter region polymorphism of the DRD4 gene with a functionally significant T variant of the –C521T polymorphism reduces transcriptional efficiency (Okuyama et al., 2001). In a meta-analysis, this –C521T polymorphism was found to be associated with novelty seeking (Schinka et al., 2002). An interesting finding was found that only the presence of the 48-bp VNTR and the –C521T polymorphism were associated with higher novelty seeking and lower harm avoidance scores (Lee et al., 2003). It shows the interaction between genotypes.

The TaqI polymorphism in DRD2 gene characteristically presents two alleles named A1 and A2. Human who carry the A1 allele showed low relative glucose metabolic rates in brain regions that are rich in DA receptors and participate in a variety of complex cognitive and motivational processes (Noble et al., 1997). The presence of DRD2 TaqI A1 allele has been associated to higher novelty seeking (Noble et al., 1998), higher decrease in anxiety and craving following the use of a DA receptor agonist (Lawford et al., 1995), and stress (Berman and Nobel, 1997).

1.5.3.2 COMT

A G/A transition found in codon 108/158 results in a valine-to-methionine substitution, Met (108/158) is coded by the low-activity (A allele), whereas Val (108/158) is coded by the high-activity variant (G allele). The A allele in COMT val-met polymorphism has been associated with schizophrenia (Kotler et al., 1999) and A allele carriers expressed their anger more outwardly, whereas G allele carriers expressed it more inwardly and reported more stage anger (Rujescu et al., 2004). The A allele in COMT

gene in is believed to relating mood disorders, schizophrenia and obsessive compulsive disorder (Lachman et al., 1996). Moreover, this functional polymorphism also participated in anxiety (Enoch et al., 2003) and panic disorder (Woo et al., 2004) in women.

1.5.3.3 MAO-A

MAO-A acts as a catalyst in the degradation of neurotransmitters like DA, serotonin, and noradrenaline. In animal studies, chemical inhibition and deletion of targeted gene for MAO-A leads to an accumulation of serotonin and norepinephrine but not DA in brain (Cases et al., 1995). Low platelet MAO activity has been associated with personality characteristics such as sensation seeking and impulsiveness (Oreland, 1993). The use of MAO-A inhibitors as antidepressants are well known (Dolle et al., 2004). A silent RFLP have been identified, Fnu4HI T→ G substitution in exon 8 on X chromosome, with the G allele associating with higher MAO-A activity (Hotamisligil and Breakefield, 1991). This G allele was associated with obsessive-compulsive disorder, particular in males with comorbid major depression (Karayiorgou et al., 1999) while the lower level of MAO-A activity allele has been found more common in probands with bipolar disorder (Preisig et al., 2000), particular in males with comorbid major depression (Karayiorgou et al., 1999), and the G allele was also associated with type II alcoholics (Parsin, 1999), while the T allele of MAOA G941T polymorphism was associated with generalized anxiety disorder (Tadic et al., 2003).

1.5.3.4 Serotonin (5-HT)

There are two predominant alleles in the polymorphic promoter region in 5-HTT gene (SLC6A4), (5-HT transporter gene-linked polymorphic region, 5-HTTLPR) of this gene: a long and a short allele with 16 and 14 repeat units, respectively. This 44bp deletion in the 5-HTTLPR was found in affecting transcription of the gene (Heils et al., 1996) and the short allele has lower activity and is associated with several psychiatric disorders and personality traits. For example, it was associated with harm avoidance personality traits (Lesch et al., 1996). Schizophrenic patients with the homozygous short alleles (deletion variant) in 5-HTTLPR scored significantly higher on "guilt feelings" and "depression" items of the Positive and Negative Syndrome Scale (PANSS), and self-rated inventories (EPI, MMPI, STAI) scores (Golimbet et al., 2004). A current study examines both association and linkage of the gene to two major anxiety-related personality measures, the harm avoidance scale on the TPQ and the neuroticism scale of the NEO-PI-R, in a sample of 148 Israeli subjects comprising 74 same-sex sibling pairs. The short allele was consistently present in higher scores on the TPQ harm avoidance scale ($P = 0.03$) and also in the same direction of NEO-PI-R neuroticism subscales of anxiety ($P = 0.03$) and depression ($P = 0.04$) (Osher et al., 2000). Another study showed that a novel allele with 13 repeat units, 23 base pairs shorter than the common short allele in the 5-HTTLPR was detected in a schizophrenic patient of Jewish Libyan origin who exhibited extreme aggressive behaviour and committed suicide after several attempts (Frisch et al., 2000). The results, however, have been inconsistent reported that there was no association in 5-HTTLPR and personality traits of Japanese population (Kumakiri et al., 1999) and this no association also happened in DRD4 VNTR exon III in novelty seeking (Ebstein et al., 1997).

It may be due to multi-gene interaction since there was an association found between 5-HTTLPR and DRD4: a significant DRD4 VNTR and 5-HTTLPR interaction was observed for harm avoidance, the subgroup with homozygous short 5-HTTLPR, 7-repeat DRD4 genotype showed a higher mean harm avoidance score (Szekely et al., 2004).

Moreover, in a study, Studies demonstrated that 5-HT1B autoreceptors plays a role in the pathology of depression, anxiety, and obsessive-compulsive disorder (Moret and Briley, 2000) and it was shown that chronic administration of tricyclic antidepressants or selective reuptake inhibitors desensitizes the presynaptic 5-HT1B autoreceptors. (Briley and Moret, 1993). Finnish antisocial alcoholics were found to have a higher frequency of one specific SNP (a G-to-C substitution at the nucleotide position 861) and more 861C alleles than Finnish control (Lappalainen et al., 1998).

1.5.3.5 Opioid receptor

An A118G nucleotide exchange in exon 1 of the OPRM1 gene causes an Asn40Asp substitution polymorphism in the receptor's extracellular domain. 39 healthy men were genotyped (A vs G) and then underwent opioid receptor blockade with Naloxone. Subjects carried the variant polymorphism had lower scores on the conscientiousness factor and associated subscales of NEO-PI-R compared to subjects expressing the common receptor (Wand et al., 2002). The main finding of this paper is that the cortisol response after naloxone challenge is different in A118G people.

1.5.4 Interaction between genes

It has been shown that gene polymorphisms in the serotonergic, dopaminergic and opioid systems have been shown to be associated with association with certain personality traits like novelty seeking, impulsivity and anxiety. However, the inconsistency observed may due to the interaction between genes. Benjamin et al. (2000) showed an association in modulation of novelty seeking by the significant interaction between DRD4 exon III and 5-HTTLPR, and between COMT val/met and 5-HTTLPR (Benjamin et al., 2000). Similarly another study showed that three-way univariate analysis of variance with the three polymorphisms as independent variables and with novelty seeking as dependent variable. The presence of the long 5-HTTLPR allele, in the presence of the COMT homozygous G allele genotype and carriers of the DRD4 exon III 7-repeat showed higher novelty seeking scores (Strobel et al., 2003).

1.6 Aim of study

There is an increasingly popular use of "club drugs" like ketamine, 'ecstasy', 'marijuana', 'ice', and marijuana in rave parties and discos among Chinese adolescents in Hong Kong these past few years. The first objective of this study is to obtain demographic and qualitative data such as pattern, frequency and reason of drug use amongst the young "club drug" users in Hong Kong. The time of first drug use, their knowledge about drugs of abuse, as well as physical, behavioural symptoms and potential for developing tolerance and dependence were also ascertained.

Since it was shown that an association exists between personality traits and substance

abuse, the second objective of this study is to ascertain whether there is a difference in personality traits between "club drug" users and those from the control population.

Genetic factors have been shown to influence personality traits like impulsive behaviour by affecting the imbalanced release of neurotransmitters in brain, thus implicating its role in addictive behaviours like smoking, gambling and drinking. The third objective of this study is to determine whether certain gene mutations may be associated with "club drug" use. The fourth objective is to examine whether these mutations may subsequently interact with personality traits thus may predispose certain individuals to "club drug" use.

Chinese "club drug" users satisfying the inclusion criteria of having tried ketamine, marijuana, 'ecstasy' or 'ice' at least once during their lifetime with no history of heroin use and no history of psychiatric illness, head trauma, unconsciousness and seizures were recruited for this study and the following parameters were examined: 1) A structure-interview using a questionnaire of 72 questions to establish demographic information, pattern, frequency, reasons and knowledge of drug use. Physical and behavioural symptoms as well as potential to develop tolerance and dependence were also ascertained. 2) With the use of the Chinese version of the SSS-V (Zuckerman, 1994) and the BIS/BAS scale (Carver and White, 1994), personality traits of the subjects and controls were ascertained and differences in personality traits between these two groups were compared. 3) Gene variants of the dopaminergic, serotonergic, and opioid receptors or transporter systems were examined amongst the "club drug" users and controls. 4) To establish whether a correlation exists between gene variants and personality traits. It is hoped that the results from this study can provide further

qualitative data regarding "club drug" use in Hong Kong. Furthermore, it is hoped that an interaction can be observed between gene variants and personality traits associated with "club drug" use.

CHAPTER TWO MATERIALS AND METHODS

2.1 Recruitment of Subjects

2.1.1. Club drug users

366 unrelated Chinese "club drugs" users (268 male, 98 female) were recruited for this study. The patients were derived from two sources: the Substance Abuse Assessment Clinic at the Kwai Chung hospital (n=83) and snowball recruitment from the six social services agencies, namely: Hong Kong Children and Youth Services, Chinese YMCA, the Hong Kong Federation of Young Groups, Hong Kong Playground Association, Caritas-HK, The Boys' and Girls' Clubs Association of Hong Kong (n=283).

Inclusion criteria for the selection of subjects being"club drug" users have to have ever used ketamine, 'ecstasy' (MDMA), marijuana or 'ice' (METH) at least once during their lifetime. Exclusion criteria being no history of heroin use, psychiatric disorders, head trauma, unconsciousness or seizures.

2.1.2. Controls

303 unrelated Chinese youngsters (193 male and 110 female) who are free of major medical and psychiatric problems were recruited. All subjects were required to fill in a questionnaire to ascertain their history of addiction behaviour. Only subjects with no history of alcohol, tobacco, or illicit drug use were included in this study.

The present study protocol has been approved by the Human Ethics Committee of The Chinese University of Hong Kong. All participants signed an informed written consent and those who were under 18, parents' written consent were obtained.

2.2 Phenotype assessment

A community-base structured-interview was conducted amongst the "club drugs" users that were recruited. Participants were asked to complete a questionnaire and 2 personality trait assessments (see 2.2.2). A remuneration of HKD$100 was paid to each participants who completed the 2-hour interview. For those who are willing to participate in the genotyping part of the project, a further remuneration of HKD$100 were given.

2.2.1 Questionnaire (Appendix 1a – Chinese version used; 1b – English translated version for reference only)

A total of 72 questions were constructed in the questionnaire. The information obtained on the drug users include: demographics, pattern of drug use, experiences of first time drug use, current drug use, effects of drug use, potential dependence/withdrawal and knowledge about drugs of abuse.

2.2.1.1 Demographics

It includes personal and family information: gender, age, educational background, occupation, housing and parent's marital status and ethnicity of parents.

2.2.1.2 Pattern of drug use

All data from this section was obtained for drug use in both Hong Kong and across the border from Hong Kong, it includes information like pattern of rave parties/ disco attendance; the types, quantity and frequency of drug use plus the amount spent on drugs. For cross-border use, the region/town that the users most frequent was ascertained.

2.2.1.3 First time drug use

Age, source and reason of first time drug use and frequency of continuous drug use were recorded.

2.2.1.4 Reason of drug use

To investigate the perceived function for the drugs used. This is classified into 5 domains according to Boys A. et al. (2001). This included 'changing mood', 'physical effects', 'social purposes', 'facilitate activity' and 'manage effects from other substances'.

2.2.1.5 Effects of drug use

Physical and behavioural effects of drug use were recorded. Questions are adapted from the Substance Abuse Assessment Questionnaires (SAAQ) by Ghodse H. ASAU/ CUHK, 10/15/97, ver 1.1.

2.2.1.6 Potential dependence/ withdrawal

Questions were about the potential dependence/ withdrawal and physical symptoms of stopping, cutting down on, or quitting drug use. Questions are adapted from the Substance Abuse Assessment Questionnaires (SAAQ) by Ghodse H. ASAU/ CUHK, 10/15/97, ver 1.1.

2.2.1.7 Knowledge about drug of abuse and psychological well-being.

Subjects were asked about the knowledge of drugs used and about the current psychological well-being of the subjects.

2.2.2 Personality assessments (Appendix 2a – Chinese version of SSS-V scale; 2b –Chinese version of BIS/BAS scale)

During the interview, apart from answering the questions of the questionnaire, subjects were also requested to complete the Chinese version of the SSS-V (Zuckerman, 1994) and the BIS/BAS tests (Carver and White, 1994). BIS/BAS was translated into Chinese and verified for this study. A total of 360 club drugs users and 303 controls of a similar age group and socio-economic background completed this section.

2.3 DNA extraction

At the time of interview, subjects who gave consent to the genotyping part of the study had 6 ml of blood drawn from the peripheral vein by a doctor or a registered

laboratory technician. Blood samples were collected into EDTA blood collecting tubes and stored at -70°C before DNA extraction. DNA was extracted from peripheral blood samples with the QIAamp DNA mini kit (QIAamp catalog no. 51106). First of all, 20μl of 20mg/μl proteinase K was transferred into the bottom of a 1.5ml microcentrifuge tube. 200μl whole blood was added to the proteinase K and mixed immediately by vortexing for 15 seconds (sec). 200μl buffer AL was added to the mixture and vortexed for 15 sec. Afterwards, the mixture was incubated at 56°C for 10 minutes (min) to lyse the cells and inactive proteinase K. 200μl absolute ethanol was added to the mixture and mixed by vortexing. All the mixture then was applied to the 2ml QIAamp spin column (collection tube) without wetting the rim and centrifuged at 6000 x g for 1 min. After centrifugation, the collection tube was place in a clean 2ml collection tube; the tube containing the filtrate was discarded. 500μl buffer AW1 was added to the column and centrifuged at 6000 x g for 1 min. The column was placed to a clean 2ml collection tube and the tube containing the filtrate was discarded. 500μl buffer AW2 was added to the column and centrifuged at 20000 x g for 3 min. The column was placed to a clean 2ml collection tube and the tube containing the filtrate was discarded. 200μl buffer AE was added to the column and incubated at room temperature for 1 min. DNA was eluted with 200μl by centrifugation at 6000 x g for 1 min.

2.4 Genotyping

The number of subjects and controls for each gene variant is different, this is largely due to the failure in some of the DNA extraction procedures.

2.4.1 G1947A, $Val^{108/158}$ Met polymorphism in the catechol-O-methyltransferase (COMT) gene

Genotyping for this variant was performed on 303 controls and 277 "club drugs" users. 50ng purified DNA was diluted into the PCR reaction mix consisting of PCR buffer (100mM Tris-HCl, pH 8.8 at 25°C; 500ml KCl; 0.8% Nonidet P40 and 15mM MgCl), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 25 pmol of each forward and reverse primer and 1U Taq polymerase (Invitrogen, Life Technologies, USA) in a total volume of 25µl. The Nla III polymorphism was detected by RFLP analysis using a forward primer: 5'- ACT GTG GCT ACT CAG CTG TG -3', and a reverse primer: 5'- CCT TTT TCC AGG TCT GAC AA -3' to amplify a 130bp PCR fragment. PCR was carried out in a thermal cycler (Perkin Elmer, PCR 9600, USA). The amplification steps were: i) denaturation at 94°C for 5 min, ii) 35 cycles of denaturation at 95°C for 45 sec, iii) annealing at 56°C for 45 sec, iv) synthesis at 72°C for 1 min and v) final elongation at 72°C for 10 min. 10µl of the 130 bp PCR product was digested with 5U Nla III (MBI Fermentas, USA, NY) restriction enzyme for at least 8 hours (hrs) at 37°C. The restriction-digested products were resolved on a 4% agarose gel stained with ethidium bromide. The fragment length of the G allele was 114bp, while the fragment length of the PCR product remained as 96bp when the A allele was present. Heterozygotes GA were indicated by the presence of both the 96bp and 114bp bands (Fig. 23).

2.4.2 T941G polymorphism in the monoamine oxidase A (MAO-A) gene

Genotyping for this variant was performed on 303 controls and 277 club drugs users, genders are analyzed separately since MAO-A gene is X-linked. 50ng purified DNA

was diluted into the PCR reaction mix consisting of PCR buffer (100mM Tris-HCl, pH 8.8 at 25°C; 500ml KCl; 0.8% Nonidet P40 and 15mM MgCl), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 25 pmol of each forward and reverse primer and 1U Taq polymerase (Invitrogen, Life Technologies, USA) in a total volume of 25µl. The SatI polymorphism was detected by RFLP analysis using a forward primer: 5'- GAC CTT GAC TGC CAA GAT -3', and a reverse primer: 5'- CTT CTT CTT CCA GAA GGC C -3' to amplify a 130bp PCR fragment. PCR was carried out in a thermal cycler (Perkin Elmer, PCR 9600, USA). The amplification steps were: i) denaturation at 94°C for 5 min, ii) 35 cycles of denaturation at 95°C for 45 sec, iii) annealing at 56°C for 45 sec, iv) synthesis at 72°C for 1 min and v) final elongation at 72°C for 10 min. 10µl of the 130 bp PCR product was digested with 5U SatI (MBI Fermentas, USA, NY) restriction enzyme for at least 8 hours (hrs) at 37°C. The restriction-digested products were resolved on a 4% agarose gel stained with ethidium bromide. The fragment length of the T allele was 130bp, while the fragment length of the PCR product remained as 65bp when the G allele was present. Heterozygotes TG were indicated by the presence of both the 65bp, 130bp bands (Fig. 24).

2.4.3 TaqI A Polymorphism of the DRD2 Gene

Genotyping for this variant was performed on 296 controls and 277 club drugs users. 50ng purified DNA was diluted into the PCR reaction mix consisting of PCR buffer (100mM Tris-HCl, pH 8.8 at 25°C; 500ml KCl; 0.8% Nonidet P40 and 15mM MgCl), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 25 pmol of each forward and reverse primer and 1U Taq polymerase (Invitrogen, Life Technologies,

USA) in a total volume of 25µl. The TaqI A polymorphism was detected by RFLP analysis using a forward primer: 5'-GCT CTA TCT CCA ACT CTC ACA-3', and a reverse primer: 5'-AAG TCT ACT CAC CTC CAG GTA-3' to amplify a 310bp PCR fragment. PCR was carried out in a thermal cycler (Perkin Elmer, PCR 9600, USA). The amplification steps were: i) denaturation at 94°C for 5 min, ii) 30 cycles of denaturation at 95°C for 30 sec, iii) annealing at 56°C for 30 sec, iv) synthesis at 72°C for 1 min and v) final elongation at 72°C for 10 min. 10µl of the 310 bp PCR product was digested with 5U TaqI (MBI Fermentas, USA, NY) restriction enzyme for at least 8 hours (hrs) at 65°C. The restriction-digested products were resolved on a 2% agarose gel stained with ethidium bromide. The fragment lengths of the A2 allele were 180 and 130bp, when the A1 allele was present, the fragment length of the PCR product remained as 310 bp. Heterozygous A1A2 allele was indicated by the presence of the 130 bp, 180bp and 310 bp (Fig 27).

2.4.4 7-repeat allele of a 48 bp repeat polymorphism (DRD4-7) in exon 3 of the dopamine D4 receptor gene (DRD4)

Genotyping this repeat was performed on 187 controls and 239 club drug users. 50ng purified DNA was diluted into the PCR reaction mix consisting of PCR buffer (100mM Tris-HCl, pH 8.8 at 25°C; 500ml KCl; 0.8% Nonidet P40 and 15mM MgCl), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 25 pmol of each forward and reverse primer and 1U Taq polymerase (Invitrogen, Life Technologies, USA) in a total volume of 25µl. 7-repeat allele of a 48 bp repeat polymorphism (DRD4-7) in exon 3 of the DRD4 gene was detected by using a forward primer: 5'-GCT GCT GCT CTA CTG GGC -3 and a reverse primer: 5'- GTG CAC CAC GAA

GAA GGG-3'. PCR was carried out in a thermal cycler (Perkin Elmer, PCR 9600, USA). The amplification steps were: i) denaturation at 94°C for 5 min, ii) 35 cycles of denaturation at 95°C for 45 sec, iii) annealing at 61°C for 45 sec, iv) synthesis at 72°C for 1 min and v) final elongation at 72°C for 10 min. The size of PCR products for 2-repeat allele is 475bp, 3-repeat allele is 523bp, 4-repeat allele is 571bp, 5-repeat allele is 619bp, and 6-repeat allele is 667 (Fig 30) and these fragments were resolved on a 4% agarose gel stained with ethidium bromide.

2.4.5 –521C/T single nucleotide polymorphism (SNP) I the promoter region of the dopamine D4 receptor gene (DRD4)

Genotyping for this variant was performed on 231 controls and 277 club drugs users. 50ng purified DNA was diluted into the PCR reaction mix consisting of PCR buffer (100mM Tris-HCl, pH 8.8 at 25°C; 500ml KCl; 0.8% Nonidet P40 and 15mM MgCl), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 25 pmol of each forward and reverse primer and 1U Taq polymerase (Invitrogen, Life Technologies, USA) in a total volume of 25µl. The NsbI polymorphism was detected by RFLP analysis using a forward primer: 5'- TCA ACT GTG CAA CGG GTG-3', and a reverse primer: 5'-GAG AAA CCG ACA AGG ATG GA-3' to amplify a 380bp PCR fragment. PCR was carried out in a thermal cycler (Perkin Elmer, PCR 9600, USA). The amplification steps were: i) denaturation at 94°C for 5 min, ii) 35 cycles of denaturation at 95°C for 45 sec, iii) annealing at 60°C for 45 sec, iv) synthesis at 72°C for 1 min and v) final elongation at 72°C for 10 min. 10µl of the 380 bp PCR product was digested with 5U NsbI (MBI Fermentas, USA, NY) restriction enzyme for at

least 8 hours (hrs) at 37°C. The restriction-digested products were resolved on a 4% agarose gel stained with ethidium bromide. The fragment length of the C allele was 380bp, while the fragment length of the PCR product remained as 228bp and 152bp when the T allele was present. Heterozygotes CT were indicated by the presence of all the 380bp, 228bp, and 152bp bands (Fig. 31).

2.4.6 G861C polymorphism in the serotonin receptor 1B (5-HT1B) gene

Genotyping for this variant was performed on 297 controls and 271 club drugs users. 50ng purified DNA was diluted into the PCR reaction mix consisting of PCR buffer (100mM Tris-HCl, pH 8.8 at 25°C; 500ml KCl; 0.8% Nonidet P40 and 15mM MgCl), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 25 pmol of each forward and reverse primer and 1U Taq polymerase (Invitrogen, Life Technologies, USA) in a total volume of 25µl. The HincII polymorphism was detected by RFLP analysis using a forward primer: 5'- GAA ACA GAC GCC CAA CAG GAC -3', and a reverse primer: 5'- CCA GAA ACC GCG AAA GAA GAT -3' to amplify a 548bp PCR fragment. PCR was carried out in a thermal cycler (Perkin Elmer, PCR 9600, USA). The amplification steps were: i) denaturation at 94°C for 5 min, ii) 35 cycles of denaturation at 95°C for 45 sec, iii) annealing at 56°C for 45 sec, iv) synthesis at 72°C for 1 min and v) final elongation at 72°C for 10 min. 10µl of the 548 bp PCR product was digested with 5U HincII (MBI Fermentas, USA, NY) restriction enzyme for at least 8 hours (hrs) at 37°C. The restriction-digested products were resolved on a 2% agarose gel stained with ethidium bromide. The fragment length of the G allele PCR products was 96bp and 452bp, while the fragment lengths of the PCR products were 96bp, 142bp and 310bp when the C allele was present. Heterozygotes GC were

indicated by the presence of the 96bp, 142bp, 310bp and 452bp bands (Fig. 26).

2.4.7 The 44 bp insertion/deletion polymorphism in the promoter region of the serotonin transporter gene (SLC6A4)

Genotyping this variant was performed on 300 controls and 271 club drug users. 50ng purified DNA was diluted into the PCR reaction mix consisting of PCR buffer (100mM Tris-HCl, pH 8.8 at 25°C; 500ml KCl; 0.8% Nonidet P40 and 15mM MgCl), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 25 pmol of each forward and reverse primer and 1U Taq polymerase (Invitrogen, Life Technologies, USA) in a total volume of 25µl. The 44 bp insertion/deletion polymorphism in the promoter region of the SLC6A4 gene was detected by using a forward primer: 5'-GAG GGA CTG AGC TGG ACA ACC AC-3and a reverse primer: 5'-GAG GGA CTG AGC TGG ACA ACC AC -3. PCR was carried out in a thermal cycler (Perkin Elmer, PCR 9600, USA). The amplification steps were: i) denaturation at 94°C for 5 min, ii) 35 cycles of denaturation at 95°C for 45 sec, iii) annealing at 61°C for 45 sec, iv) synthesis at 72°C for 1 min and v) final elongation at 72°C for 10 min. The size of PCR products for the long allele is 528bp and the short allele is 484bp (Fig. 29); and these fragments were resolved on a 4% agarose gel stained with ethidium bromide.

2.4.8 T921C Polymorphism in Exon 3 of the Human DOR (hDOR) Gene

Genotyping for this variant was performed on 277 controls and 303 club drugs users. 20ng purified DNA was diluted into the PCR reaction mix consisting of PCR buffer (100mM Tris-HCl, pH 8.8 at 25°C; 500ml KCl; 0.8% Nonidet P40 and 15mM MgCl),

1.5 mM $MgCl_2$, 0.2 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 25 pmol of each forward and reverse primer and 1U Taq polymerase (Invitrogen, Life Technologies, USA) in a total volume of 25µl. The T921C polymorphism was detected by an artificial BstEII RFLP analysis. Using a forward primer: 5'-TTC GTC ATC GTC TGG ACG CT-3' and a modified reverse primer: 5'-GGT TGA GGC TGC TAT TGG **G**GT A-3' in which nucleotide 1158 (underlined) was changed from a C to a G, thereby an artificial BstEII restriction site was generated. These two primers were combined to amplify a 106bp PCR fragment. PCR was carried out in a thermal cycler (Perkin Elmer, Model no. PCR 9600, USA). The amplification steps were: i) denaturation at 94°C for 5 min, ii) 35 cycles of denaturation at 94°C for 45 sec, iii) annealing at 62°C for 45 sec, iv) synthesis at 72°C for 45 sec and v) final elongation at 72°C for 7 min. 8µl of the 106 bp PCR product was digested with 5U BstEII (MBI Fermentas, USA, NY) restriction enzyme overnight at 37°C. The restriction digest products were resolved on a 5% agarose gel stained with ethidium bromide. The fragment length of the T allele was 89 bp, while the fragment length of the PCR product remained as 106 bp when the C allele was present. Heterozygotes TC were indicated by the presence of both the 89 and 106 bp bands (Fig. 25).

2.4.9 A118G polymorphism in Exon 1 of the Human MOR (hMOR) Gene

Genotyping for this variant was performed on 298 controls and 267 club drugs users. 20ng purified DNA was diluted into the PCR reaction mix consisting of PCR buffer (100mM Tris-HCl, pH 8.8 at 25°C; 500ml KCl; 0.8% Nonidet P40 and 15mM MgCl), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 25 pmol of each forward and reverse primer and 1U Taq polymerase (Invitrogen, Life Technologies,

USA) in a total volume of 25µl. The A118G polymorphism was detected by an artificial Bsp681 RFLP analysis. Using a forward primer: 5'- TCA ACT TGT CCC ACT TAG ATC G -3' and a modified reverse primer: 5'- ACG CAC ACG ATG GAG TAG AG -3' in which nucleotide 353 (underlined) was changed from a G to a C, thereby an artificial NruI restriction site was generated. These two primers were combined to amplify a 151bp PCR fragment. PCR was carried out in a thermal cycler (Perkin Elmer, PCR 9600, USA). The amplification steps were: i) denaturation at 94°C for 5 min, ii) 35 cycles of denaturation at 94°C for 45 sec, iii) annealing at 62°C for 30 sec, iv) synthesis at 72°C for 45 sec and v) final elongation at 72°C for 7 min. 8µl of the 151 bp PCR product was digested with 5U NruI (MBI Fermentas, USA, NY) restriction enzyme overnight at 37°C. The restriction digest products were resolved on a 5% agarose gel stained with ethidium bromide. The fragment length of the A allele was 151bp, while the fragment length of the PCR product remained as 22bp and 129bp when the G allele was present. Heterozygotes AG were indicated by the presence of the 22bp, 129bp and 151bp bands (Fig. 28).

2.5 DNA sequencing

DNA sequencing was employed to ensure the accuracy of the genotype obtained. The PCR Clean Up system (NucleoSpin® Extract kit, Macherey-nagel, Catalog no. 740590.250, Germany) was used. The 25 μl PCR product was pipetted to a 1.5ml tube and 100 μl of buffer NT2 was added. After mixing well, the mixture was transferred to the NucleoSpin® Extract column into a 2 ml collecting tube and then centrifuged for 1 min at 11,000g. The flow-through was discarded and the column

was placed back into the 2ml collecting tube. 600 μl buffer NT3 was added into the column and then centrifuged for 1 min at 11,000g. The filtrate again was discarded and the column and then placed back into the 2ml collecting tube. Further 200 μl buffer NT3 was added and then centrifuged for 2 min at 11,000g to remove buffer NT3 quantitatively. Finally, the column as placed into a new 1.5ml tube and then 50 μ l elution buffer NE was added to the center of the column. The tube was left to stand at room temperature for 1 min and DNA was eluted by centrifugation for 1 min at 11,000g. 5 μl of purified PCR product was mixed with 1 μl 3.2 pmol/ μl forward primer, 8 μl terminator ready reaction mix (ABI PRISM® BigDye™ Terminators V 3.1 Kit, Perkin Elmer Applied Biosystems, USA), and 6 μl deionized water were mixed into a 20 μl mixture. The sequencing reaction was carried out at 96°C for 10 sec, then 25 cycles for 96°C for 10 sec, 50°C for 5 sec, 60°C for 4 min and finally 4°C for 7 in. Prior to DNA sequencing, an AutoSeq™G-50 column (Amersham Pharmacia Biotech Inc, USA) was used to remove the excess dRhodamine Terminator from the completed DNA sequencing reaction. DNA sequencing was then performed in an automated ABI PRISM® 3700 DNA Analyzer (Perkin Elmer Applied Biosystems, USA).

2.6 Statistical Analysis

Data will be analysed using SPSS (version 11) for Windows. Descriptive statistics like mean (SD) and percentages will be used to summarize the information like demographic, patterns and extent of drug use.

For the personality trait tests, Chronbach's alpha coefficient was used to initially

determine the internal reliability of the personality trait subscales. The mean scores of SSS-V and BIS/BAS subscales between subjects and controls were compared using the Student's t-test.

2-tailed Yates chi-square (χ^2) analysis was applied to compare the difference in allele and genotype frequencies between (1) the club drug users and the controls, (2) prevalence of club drug by gender. $P< 0.05$ was considered statistically significant.

Multivariate analysis with Bonferroni correction was used to study the interaction of personality traits subscales scores and each genotype. Comparisons between all three genotype groups of each candidate gene variant with mean scores of SSS-V and BIS/BAS subscales were carried by one-way ANOVA. The mean scores of SSS-V and BIS/BAS subscales were compared between subsamples defined by the presence or absence of alleles of each candidate gene variant by using Student's t-test.

CHAPTER THREE RESULTS

3.1 Results from questionnaire

3.1.1 Demographics of club drug users

3.1.1.1 Gender and age

Of the 336 club drugs users who were interviewed, 73.2% were male and 26.8% were female (Fig. 1). The age range of the respondents is 13 to 29 with a mean age of 18.12 ±2.48 years. The subjects mainly fall in the age range of 15 to 20 years (Fig. 2).

3.1.1.2 District of residence

87.8% club drugs users lived in the New Territories, 11.4% lived in Kowloon, and 0.8% lived on Hong Kong Island in this study. The 5 most commonly districts of residence being Shatin (23.4%), Tsuen Wan (18.5%), Tai Po (16.9%), Kwai Tsing (15.2%) and North District (8.6%) (Table 1).

3.1.1.3 Type of living quarters and cohabitation

Among 336 club drugs users, 73.2% lived in public housing and 16.9% in private housing, and the remaining in other types of housing such as rented private house and home ownership estates (Fig. 3). 68.8% lived with their parents whilst 23.3% lived with their single parent 23.2%; the remainder live with relatives, friends or they live alone (Fig. 4).

3.1.1.4 Educational attainment and employment status

On the educational background of the club drugs users, 58.7% has lower secondary education and 37.2% had upper secondary education. Most of the subjects had Form 3 level (34.7%) followed by Form 5 level (21.9%) of education (Fig. 5). Information on their employment status showed that 42.1% subjects were unemployed, 30.9% were still employed (either part-time or full-time), and 21% were students (Fig. 6).

3.1.1.5 Parents' details

The parent's marital status was usually normal (61.9%) among the club drug users with only 38.1% were separated, divorced, re-married or with either of the parent deceased (Fig. 7). Most of the respondent's father had full time jobs (59%) and only 13.9% were unemployed while 39.5% of the respondent's mothers have full time job (with 38.6% of them being housewives (Fig. 8). The educational background of the subject's parents was mainly primary school and secondary school level (Fig. 9). More mothers had primary school educational background than those in fathers' (42.7% vs 34.7%) while more fathers had secondary school educational background than those in mothers' (35.2% vs 30.4%) (Fig. 9). More than half of the subjects' fathers had addictive habits like smoking (54.9%), drinking (36.1%), gambling (33.6%) and taking drugs (2.7%) (Fig. 10) while less than 10% of these addictive behaviours were shown in the mothers (Fig. 11).

Fig. 1) Demographics - Gender of 366 club drugs users.

Table. 1) Demographics - District of residence of 366 club drug users.

Fig. 1 Gender of club drugs users

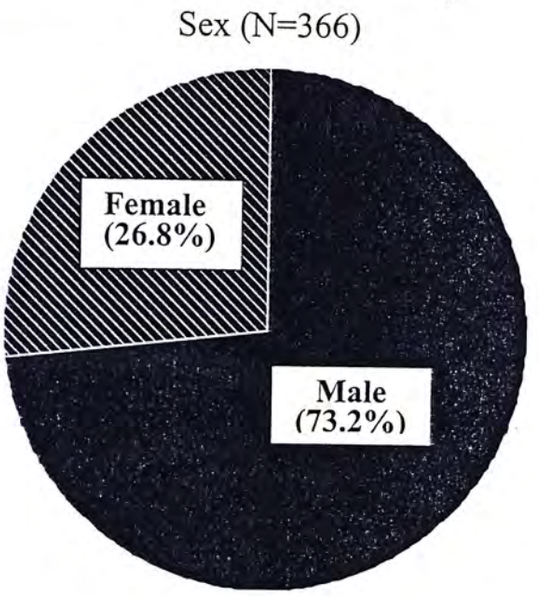


Table 1. District of residence

(N=366)	%
Hong Kong Island	**0.8**
Central & Western	0
Wan Chai	0
Eastern	0.5
Southern	0.3
Kowloon	**11.4**
Yau Tsim Mong	2.1
Sham Shui Po	3.3
Kowloon City	0.3
Wong Tai Sin	3.8
Kwun Tong	1.9
New Territories & Island	**87.8**
Kwai Tsing	15.2
Tsuen Wan	18.5
Tuen Mun	2.5
Yuen Long	0.8
North District	8.6
Tai Po	16.9
Sha Tin	23.4
Sai Kung	1.4
Islands	0.5

Fig. 2) Demographics - Stem-and-Leaf Plot of age of 366 club drugs users.

Fig. 3) Demographics - Type of living quarters of 366 club drug users.

Fig. 2 Stem-and-Leaf Plot of age in club drugs users

```
 Frequency     Stem &  Leaf

    1.00       13 .  0
    9.00       14 .  0000
   29.00       15 .  00000000000000
   54.00       16 .  000000000000000000000000000
   79.00       17 .  0000000000000000000000000000000000000000
   63.00       18 .  000000000000000000000000000000
   44.00       19 .  00000000000000000000
   34.00       20 .  00000000000000000
   14.00       21 .  0000000
   21.00       22 .  0000000000
    6.00       23 .  000
   12.00 Extremes    (>=24.0)

Stem width: 1
Each leaf: 2 case(s) & denotes fractional leaves.
```

Fig. 3 Type of living quarters

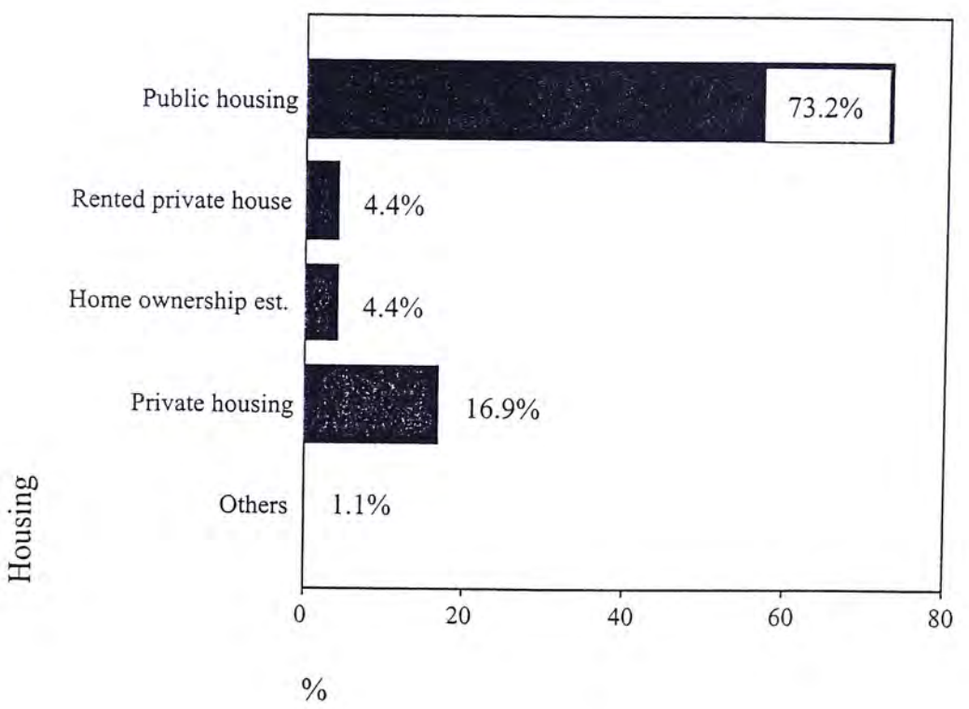


Fig. 4) Demographics - Cohabitation of 366 club drugs users.

Fig. 5) Demographics - Educational attainment of 366 club drugs users.

Fig. 4 Cohabitation of club drugs users

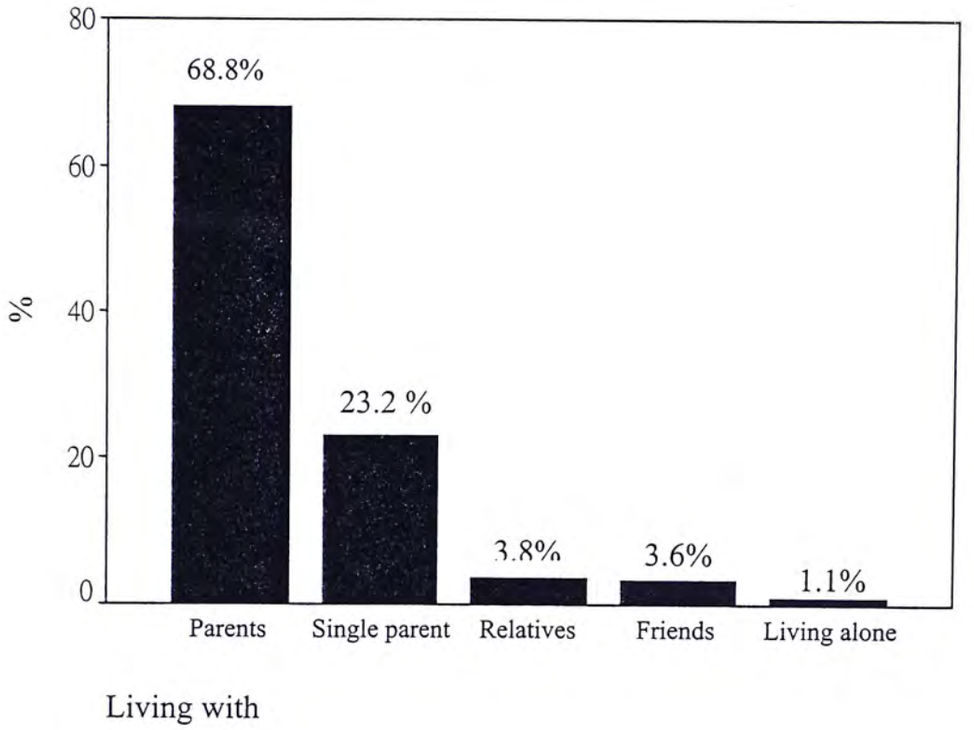


Fig. 5 Educational attainment of club drugs users

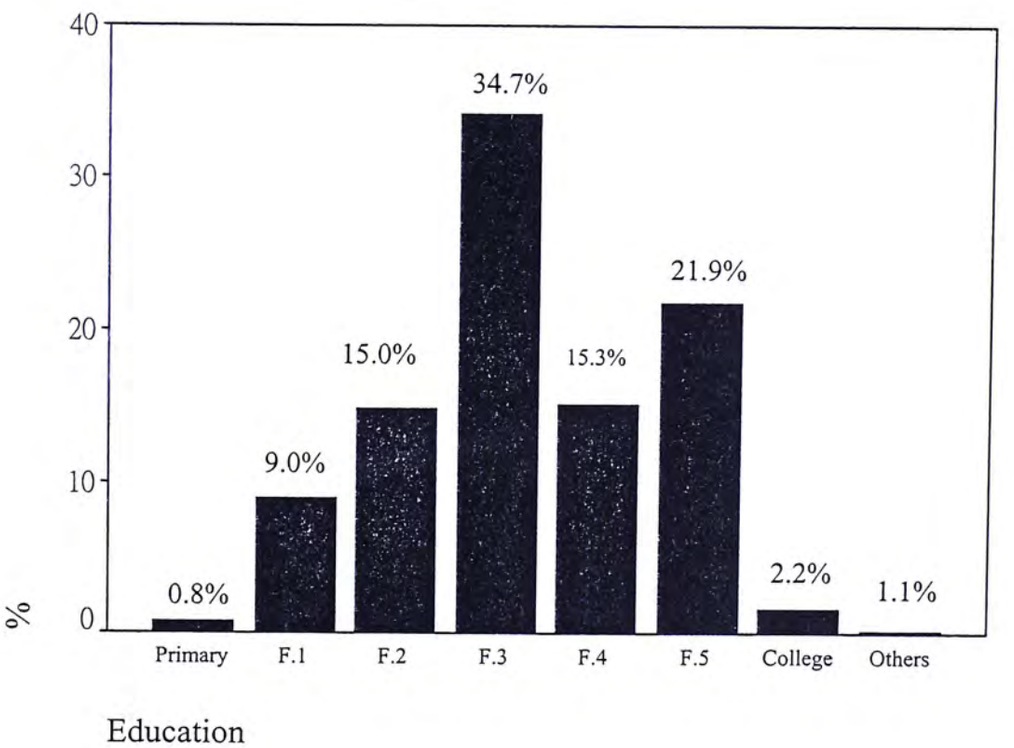


Fig. 6) Demographics - Occupation of 366 club drugs users.

Fig. 7) Demographics - Parental marriage status of 366 club drugs users.

Fig. 6 Occupation of club drugs users

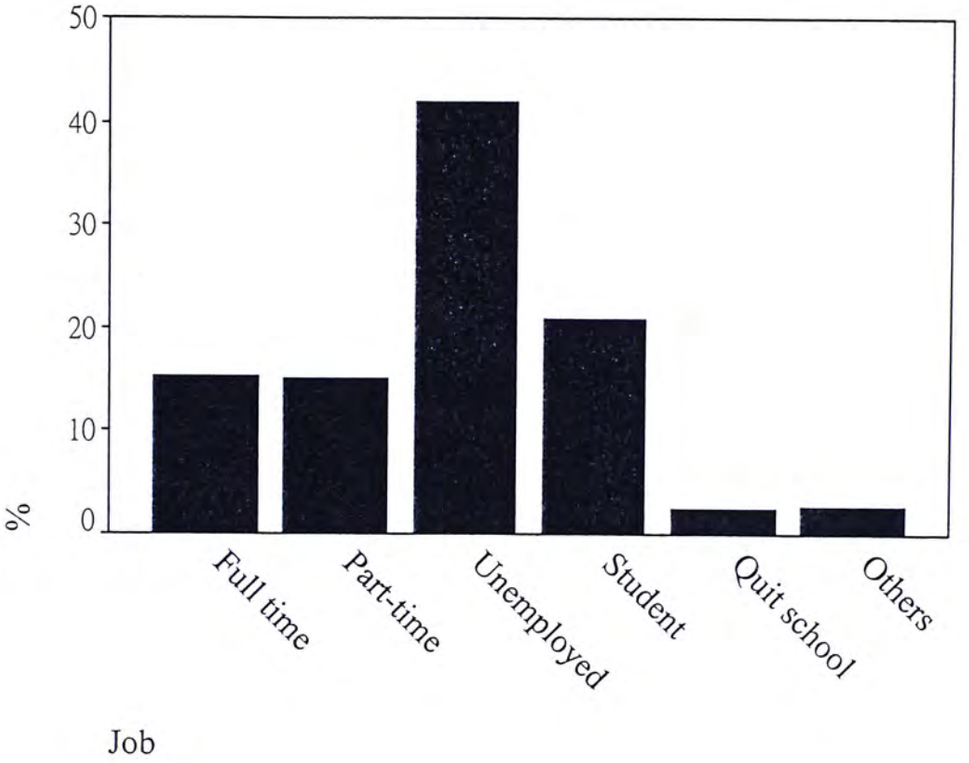


Fig. 7 Parental marriage status

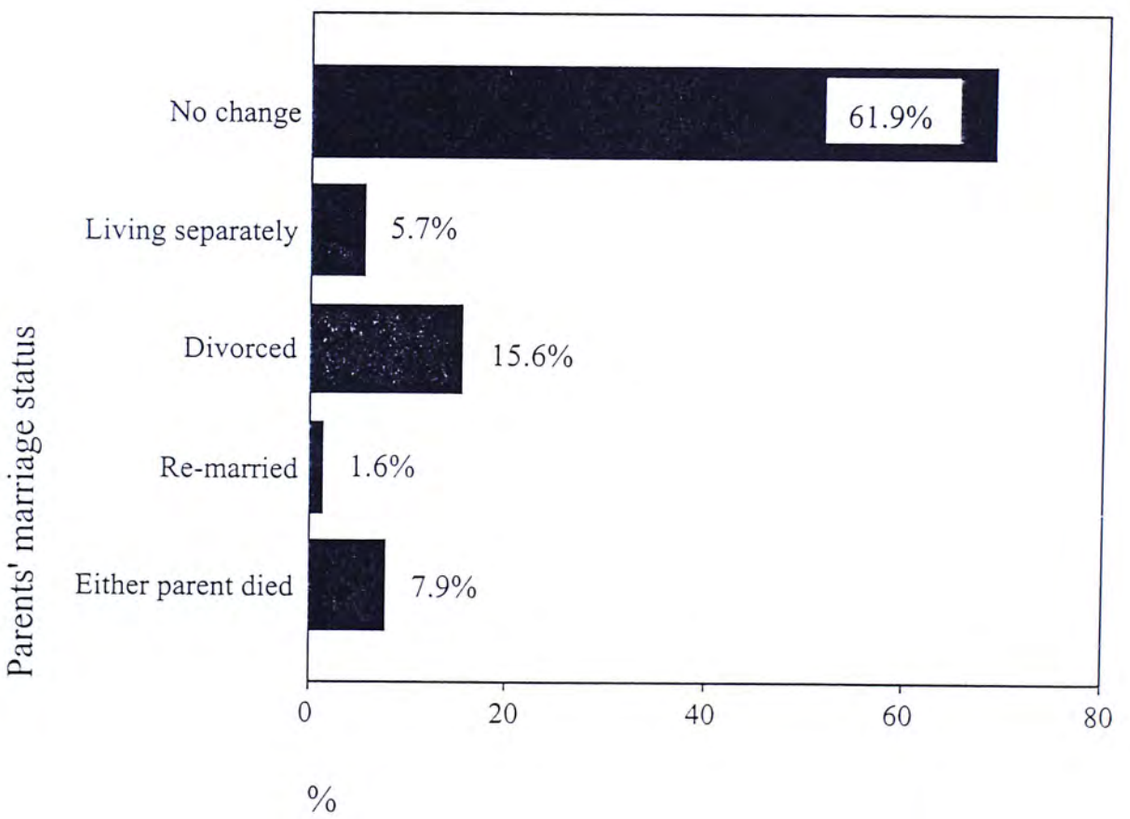


Fig. 8) Demographics - Parental working status of 366 club drugs users.

Fig. 9) Demographics - Parental educational level of 366 club drugs users.

Fig. 8 Parental working status

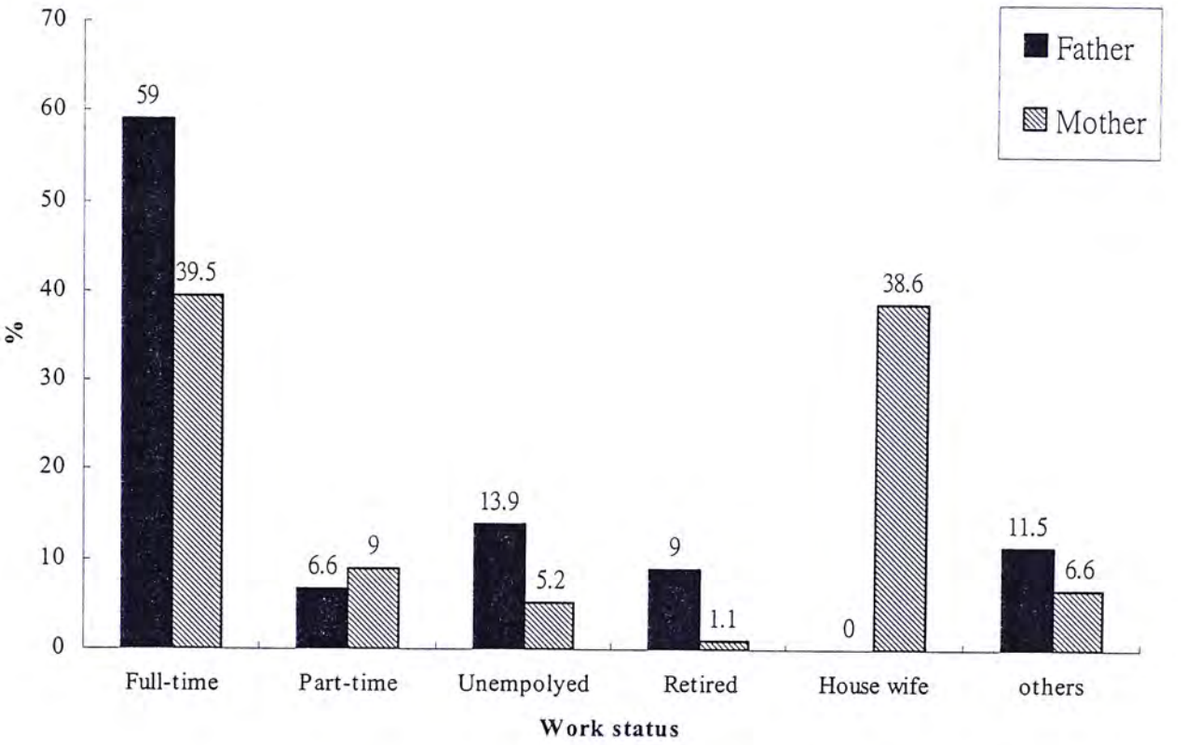


Fig. 9 Parental educational level

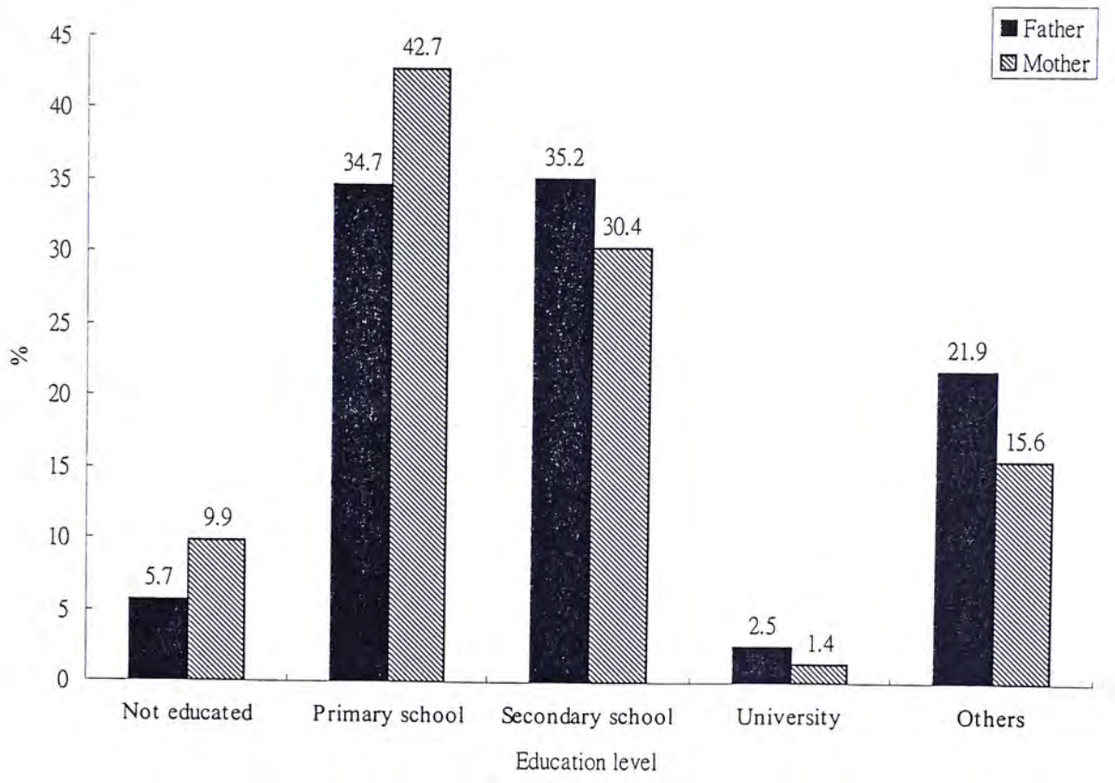


Fig. 10) Demographics - Father's habits including smoking, gambling, drinking and drug use.

Fig. 11) Demographics - Mother's habits including smoking, gambling, drinking and drug use.

Fig. 10 Father's habits

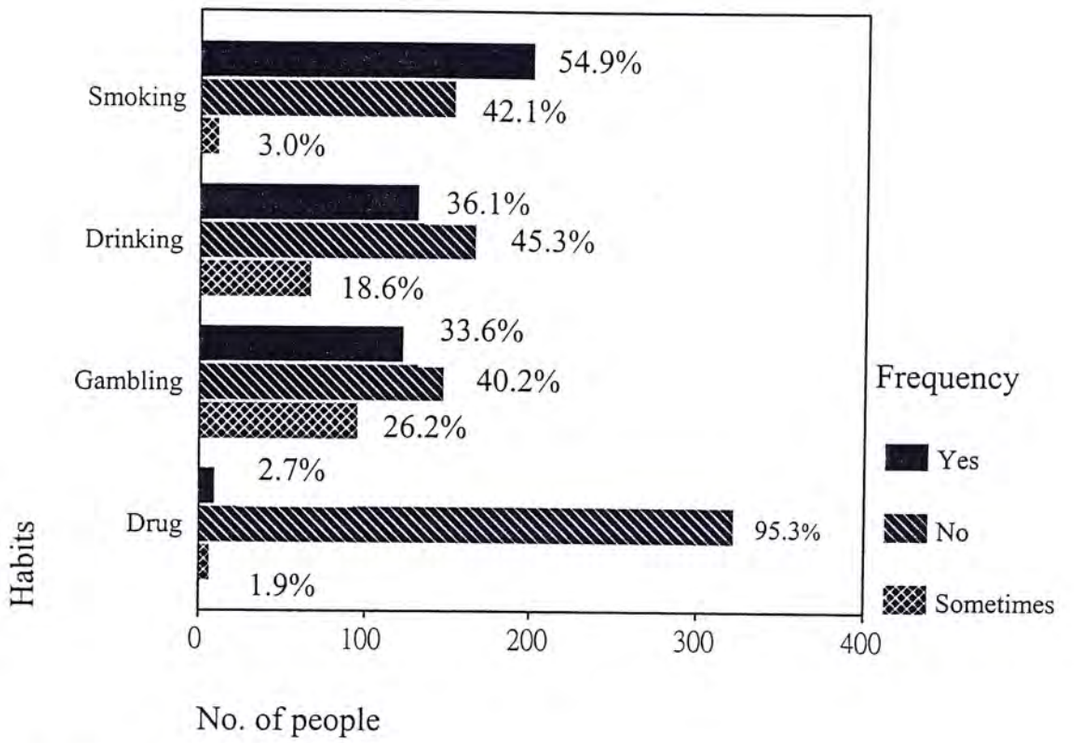
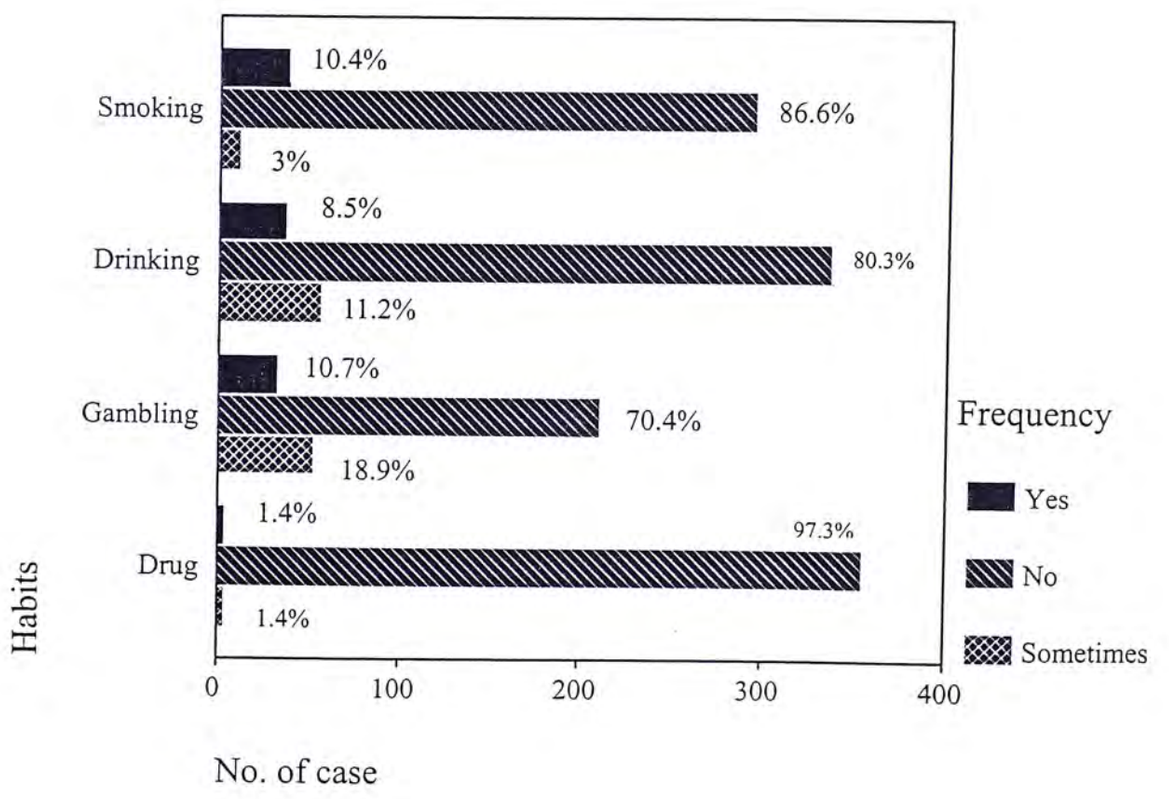


Fig. 11 Mother's habits



3.1.2 First time drug use

The mean age of initial drug use is 14.8±2.2 years old (range: 10-20 years). Subsequent to their initial drug use, 34% of club drug users took drugs 1 to 3 times per month, 24% took drugs 1 to 6 times per week and 24.3% took drugs occasionally without a specific drug use pattern (Fig. 12).

When they tried drugs for the first time, their main source of drugs came from friends (79.4%) followed by friends in triad society (36%) and drugs dealers in discos or rave parties (28.6%) (Fig. 13).

The reasons for the first time drug use are 'peer pressure' (62.6%), 'curiosity' (58%), 'to seek excitement and fun' (55.2%) and 'to relieve boredom' (40.1%) (Fig.14).

Fig. 12) First time drug use - Frequency of drugs taking subsequent to initial drug use of 366 club drugs users.

Fig. 13) First time drug use - Source of drugs during first time drug use of 366 club drugs users.

Fig. 12 Frequency of drugs taking subsequent to initial drug use

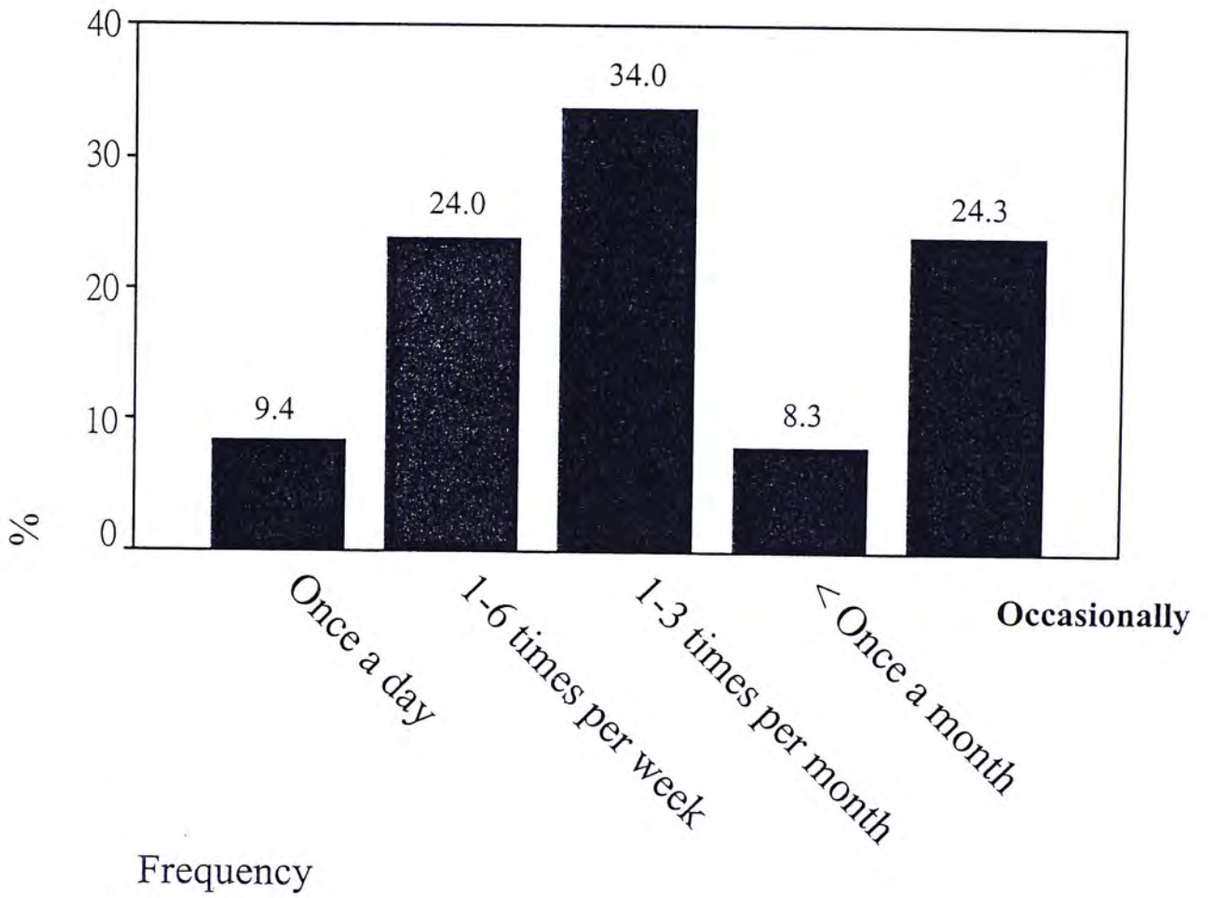


Fig. 13 Source of drugs during first time drug use

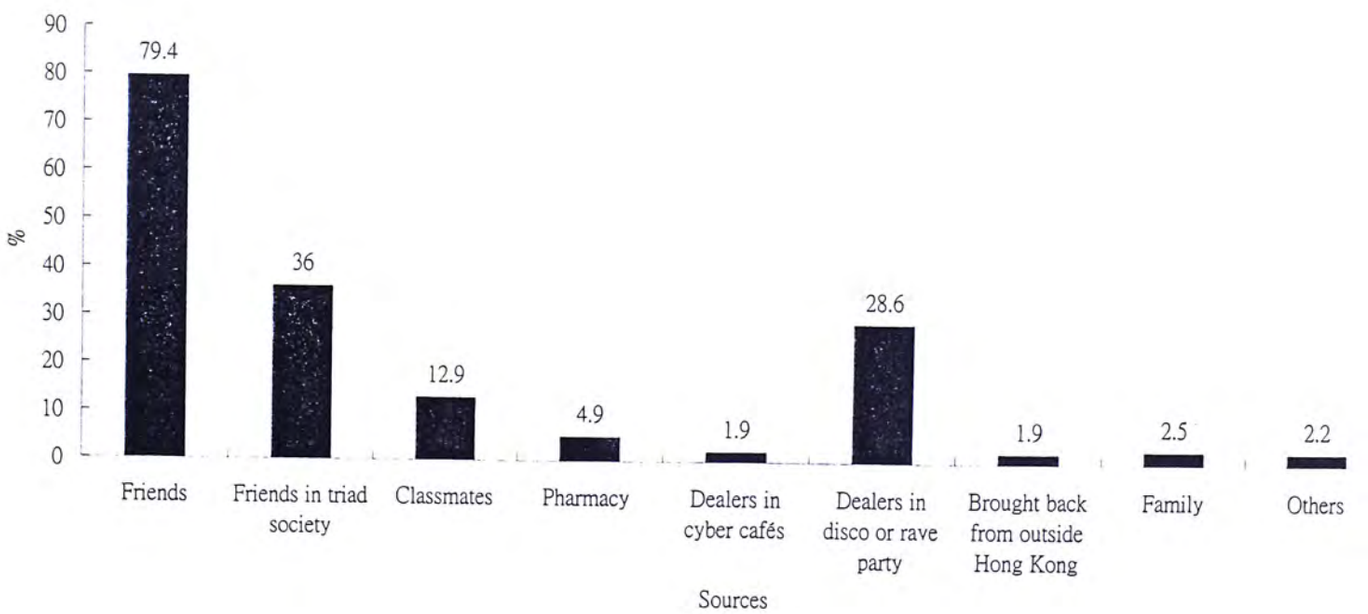
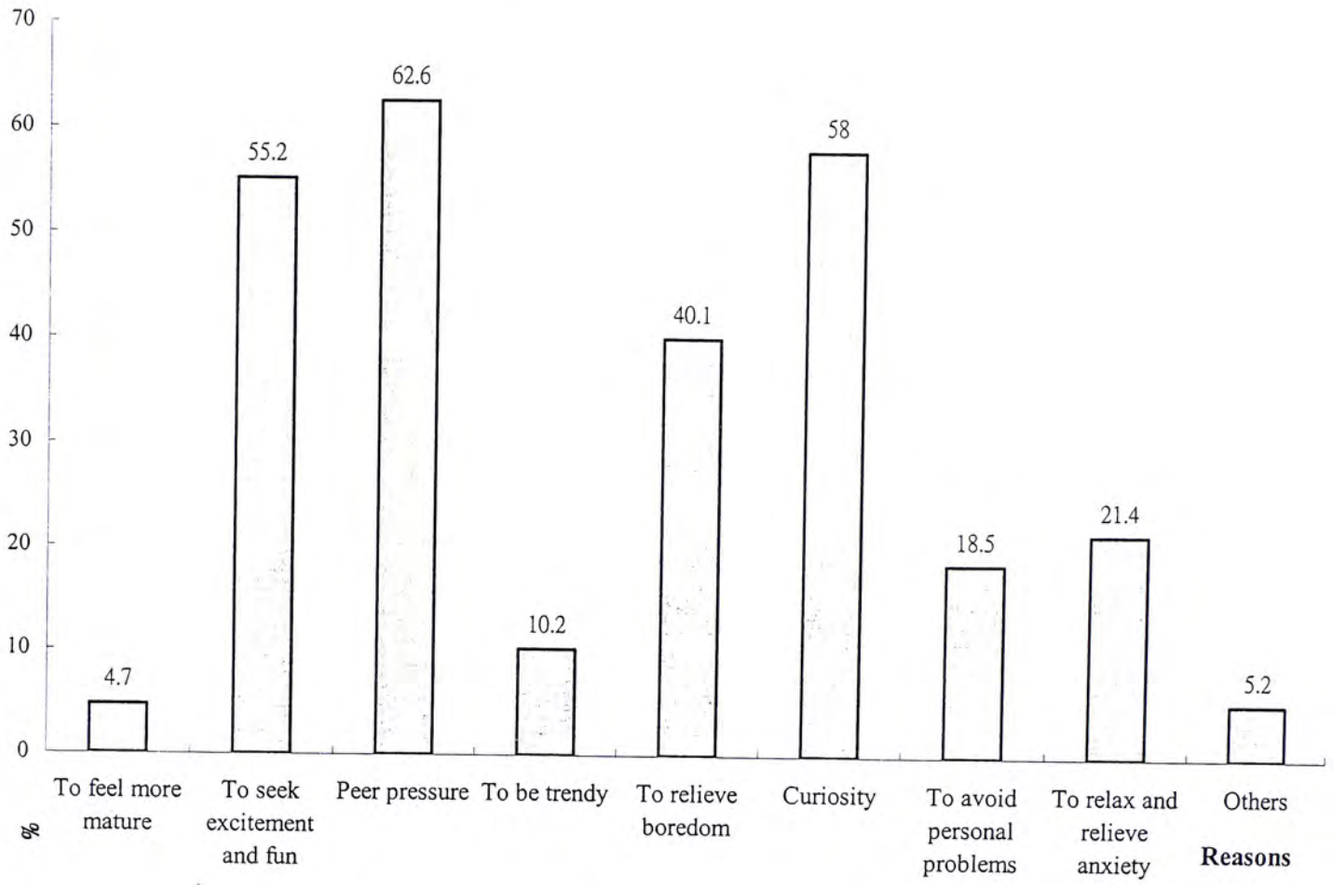


Fig. 14) First time drug use - Reasons of first time drug use of 366 club drugs users.

Fig 14. Reasons of first time drug use



3.1.3 Prevalence of drug use

The types of drugs used were similar for the periods: past 30 days (current use), past 12 months or lifetime use in the order from the most prevalent (excluding alcohol and cigarette). They are ketamine, marijuana, 'ecstasy' and 'ice' (Table 2). The commonest type of drugs that club drugs users have ever tired was ketamine (88.5%) followed by marijuana (83.6%), 'ecstasy' (79.8%), ice (30.1%), benzodiazepines (24.0%), cough mixture (21.6%) and solvents (10.1%). The prevalence of club drugs used within the past year is: ketamine (81.1%), followed by marijuana (78.4%), 'ecstasy' (69.9%), benzodiazepines (24.0%), cough mixture (21.6%), 'ice' (18.6%), and solvents (10.1%). The prevalence of current drugs use is: ketamine (54.4%), followed by marijuana (44.4%), 'ecstasy' (39.1%), cough mixture (9.3%), 'ice' (8.2%), benzodiazepines (4.1%) and solvents (2.2%).

Of all club drug users interviewed in this study, 85.2% and 83.6% have smoked or consumed alcohol in their lifetime respectively. In the past year, 83.1% and 80.9% of them have smoked or drank alcohol respectively and in the current drug users, 71.2% and 65.2% of them still smoked or drank alcohol respectively.

When male and female club drug users were examined separately, it is evident that the prevalence of marijuana and 'ice' used was significantly different (Table 2). Males showed a significantly higher prevalence of lifetime (OR=1.91, p=0.04) and last 12 months (OR=1.97, p=0.017) use of marijuana. However, females showed a significantly higher prevalence of lifetime (OR=2.21, p=0.002) and last 12 months (OR=2.28, p=0.005) use of 'ice'.

3.1.4 Frequency and quantity of drug used

It can seen that the mean no. of days (± S.D.) of drugs used in the last 30 days for each club drug used was shown in Table 3 with cigarette (21.6±8.35) and alcohol (7.5±1.1) being the most frequently used followed by marijuana (3.9±3.7), ketamine (3.2±2.9), 'ecstasy' (3.1±2.8) and cough mixture (3.2±0.37).

The average quantity of each club drug used at any one occasion was shown in Table 3. 70 to 100% of the users had 2 units of ketamine, 'ecstasy' or marijuana; 1 unit of 'ice', cigarette or cough mixture; 8 units of alcohol or benzodiazepines; only 0.5 unit for solvents at any one occasion.

Table. 2) Prevalence of club drug use among 366 club drug users during the periods of lifetime, the last 12 months, and the last 30 days in Hong Kong.

Table 2. Prevalence of drug use in Hong Kong

	Group	Lifetime (%)	Last 12 Months (%)	Last 30 Days (%)
Ketamine	Total	88.5	81.1	54.4
	Male	87.7	80.2	53.0
	Female	90.8	83.7	58.2
Ecstasy	Total	79.8	69.9	39.1
	Male	77.6	67.2	35.8
	Female	85.7	77.6	48.0
Marijuana	Total	83.6	78.4	44.4
	Male	86.2*	81.7*	46.1
	Female	76.5	69.4	39.8
Ice	Total	30.1	18.6	8.2
	Male	25.4	14.9	7.1
	Female	42.9*	28.6*	11.2
Alcohol	Total	83.6	80.9	65.2
	Male	85.8	82.8	67.4
	Female	77.6	75.5	58.2
Cigarette	Total	85.2	83.1	71.2
	Male	87.3	84.7	73.0
	Female	79.6	78.6	66.3
Benzodiazepines	Total	24.0	14.2	4.1
	Male	24.3	13.1	4.9
	Female	23.5	17.3	2.0
Cough Mixture	Total	21.6	15.6	9.3
	Male	24.3	18.4	11.2
	Female	14.3	8.2	4.1
Solvents	Total	10.1	3.3	2.2
	Male	10.1	3.0	1.5
	Female	10.2	41	4.1

* Significant difference by gender in a specific period of drug use. (Yate's χ^2 analysis)

Table. 3) Frequency and quantity

Results showed i) the no. of club drugs users who have ever tried a specific club drug; ii) the no. of days per months (last 30 days) in which a specific club drug was consumed (mean ± S.D); iii) the quantity of drug use as ≤unit (as specify with each drug used) and the percentage of users using this quantity shown in brackets.

Table 3. Frequency and quantity of drugs used

Type of drug	No. of users for each drug	Frequency, days on drugs in the last month (mean±SD)	Quantity ≤Unit, (%)
Ketamine	199	3.2±2.9	2/pack, (89.4)
Ecstasy	143	3.1±2.8	2/tablet, (95.5)
Marijuana	163	3.9±3.7	2/joint, (86.2)
"Ice"	30	1.7±0.1	1/row, (78.5)
Alcohol	239	7.5±1.1	8/can, (79.2)
Cigarette	304	21.6±8.35	1/pack, (85.5)
Benzodiazepines	52	1.2±0.02	8/tablet, (71.8)
Cough Mixture	57	3.2±0.37	1/bottle, (100)
Solvents	12	2.5±0.54	0.5/bottle, (73.1)

3.1.6 Pattern of polydrug use

The most frequent types of drug used amongst the subjects were ketamine (55.6%), 'ecstasy' (21.5%), marijuana (14.6%) and 'ice' (3.6%) (Fig. 15). 285 (77.9%) club drug users with 201males (75% of total male subjects) and 84 females (85.7% of total female subjects) being polydrug users in which 165 (45.1%) drug users took 3 types of drugs at any one occasion whereas 120 (32.8%) of them took 2 types of drugs at any one time. Ketamine, 'ecstasy' and marijuana were the most popular drugs used together with 126 (44.2%) of them using these 3 drugs together at any one occasion. Second to this, ketamine and 'ecstasy' were the most popular choice in the 2 types of drugs used together with 85 (29.8%) of them using these 2 drugs at any one occasion. Only 81 (22%) club drug users have never tried polydrug use at any one occasion of which 49 (13.4%) were ketamine users followed by 19 (5.2%) 'ecstasy' users (Table 4).

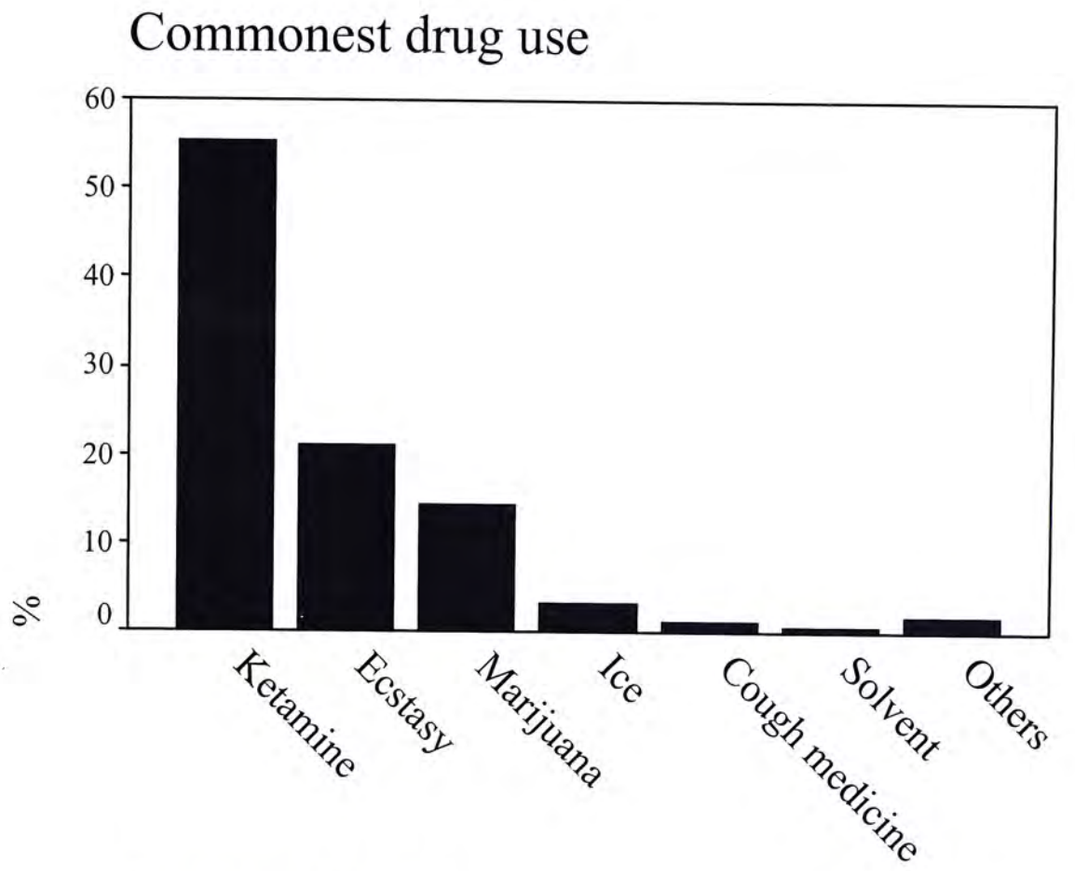
3.1.7 Drug spending

90.3% club drug users spent HK$2000 (US$253) or less per month on drugs with 71.3% of these drug users having an income of HK$4000 (US$506) or less. Financial source are from salary (32%), family (28.6%), and 'free' (24.1%) for males (Fig. 16). However, for females, 41.8% of them obtain their drug for free (significantly different to males with OR=2.25, 1.34> CI> 3.77, p=0.001) usually at rave parties or discos. Their other financial sources come from family in the form of pocket money (30.6%) and salary (16.3%) (Fig. 16).

Fig. 15) Common types of drug used among 366 club drugs users.

Table. 4) Pattern of polydrug use including the pattern of non-mixing drug use and the combination of 2 types or 3 types of drug uses.

Fig. 15 Common types of drug used



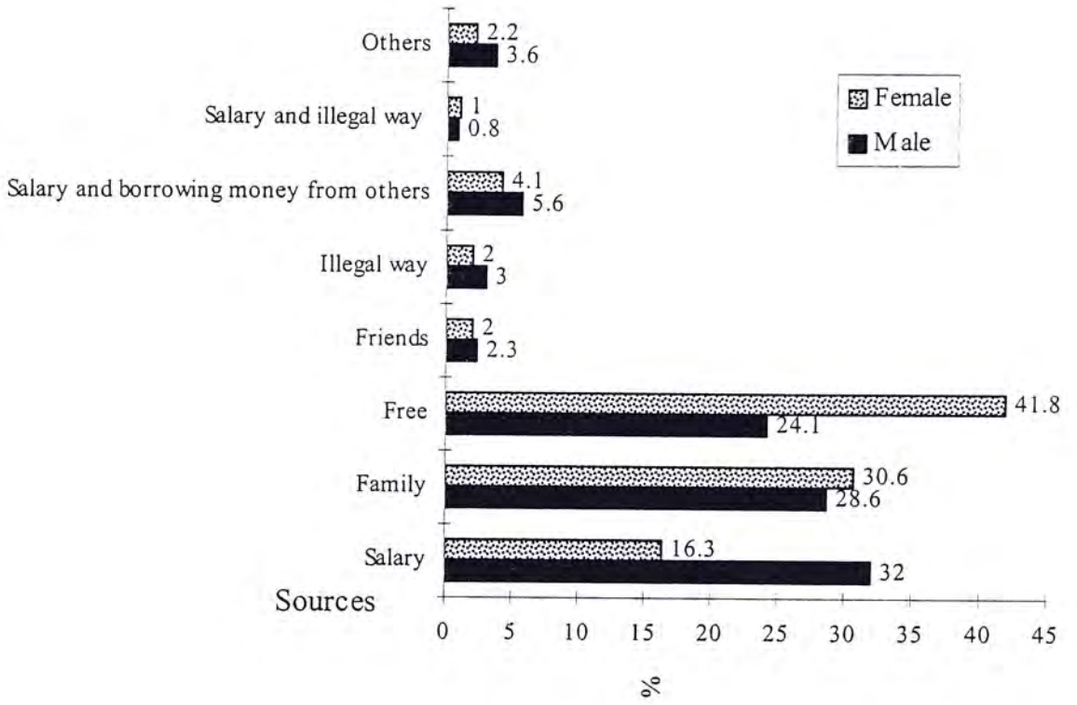
Commonest drug use

Table 4. Pattern of polydrug use

	Drugs	N	%
1 Type of drug	Ketamine	49	**13.4%**
	'Ecstasy'	19	5.2%
	Marijuana	8	2.2%
	Cough Medicine	3	0.8%
	'Ice'	1	0.3%
	Solvents	1	0.3%
2 Types of drug	Ketamine + 'Ecstasy'	85	**23.2%**
	Others	35	9.6%
3 Types of drug	Ketamine + 'Ecstasy' + Marijuana	126	**34.4%**
	Others	39	10.6%
Total		366	100%

Fig. 16) Financial sources in obtaining the drugs by gender.

Fig. 16 Financial sources in obtaining the drugs



3.1.8 Pattern of drug use in and outside Hong Kong

In Hong Kong, 311 (85%) club drugs users attended rave parties or discos in Hong Kong of which 239 (76.8%) have been to rave parties or disco within the last six months. 91.7% of those who had attended rave parties or discos have ever tried drugs (other than alcohol and cigarettes).

212 (57.9%) have been to rave parties or discos outside of Hong Kong with 147 (40.2%) went there within the last six months. Shenzhen (199, 54.4%) was the most popular place that club drug users usually have rave parties or discos outside of Hong Kong of which all the club drug users showed that they have ever tried drugs (other than alcohol and cigarettes) whilst attending rave parties and discos in Shenzhen (Fig. 17).

When the drug use pattern of the club drug users were examined, they were further classified into two major subgroups, i) those who are cross-border drug users and ii) those who are non cross-border users. (Since approximately 85% of the cross-border club drug users go to Shenzhen, only those figures of drug use in Shenzhen are reported here). The pattern of drug use of the cross-border users was further classified into a) drug use in Hong Kong and b) drug use in Shenzhen. This is to ascertain 1) whether there is a difference in the pattern of drug use across the border to that in Hong Kong, and 2) whether there is a difference in drug use pattern between the cross-border drug users and the non cross-border drug users. The pattern of drug use was further classified into the period of lifetime use, drug use in the last 12 months and in the last 30 days (current use) of (Table 5).

Results from the present study showed that there are 154 (42.1%) non cross-border drug users and 199 (57.9%) cross-border drug users. When cross-border (Hong Kong) and non cross-border (Hong Kong) drug using pattern was compared, cross-border drug users in Hong Kong use a similar or higher amount of all types of drugs when compared to non cross-border drug users in Hong Kong (Table 5). This prevalence is significantly higher for lifetime marijuana (OR=2.18, p=0.001) and benzodiazepines use (OR=2.58, p=<0.001). For drug use in the last 12 months, a significantly higher prevalence of 'ecstasy' (OR=1.91, p=0.037) and benzodiazepines use (OR=2.28, p=0.018) was found between the cross-border drugs users and the non cross-border drug users in Hong Kong.

When cross-border users who use drugs in Hong Kong and Shenzhen were compared, it can be observed that there is a slightly higher prevalence of lifetime use and the last 12 months use for club drugs like ketamine and 'ecstasy' in Shenzhen than in Hong Kong. In general, cross-border club drug users had higher lifetime drug use in prevalence of all kind of drugs listed in Table 4 except for the drugs ketamine and 'ecstasy' in Hong Kong than that in Shenzhen. This prevalence is significantly higher for lifetime marijuana (OR=1.95, p=0.015), 'ice' (OR=4.39, p<0.0001), benzodiazepines (OR=2.45, p=0.0002), cough mixture (OR=3.20, p<0.0001) and solvent use (OR=7.71, p=<0.0001). For drug use in the last 12 months, a significantly higher prevalence of marijuana (OR=2.12, p=0.0016) and 'ice' use (OR=2.21, p=0.009) was found between the cross-border drugs users in Hong Kong and the cross-border drug users in Shenzhen. However, for current cross-border drug users, the prevalence of ketamine (OR=1.88, p=0.002), marijuana (OR=2.43, p<0.0001),

alcohol (OR=3.28, p<0.0001) and cigarette (OR=3.05, p<0.0001) use in Hong Kong were significantly higher than that of Shenzhen (Table. 5).

216 (59%) and 135 (37%) club drugs users interviewed recount their having attended rave parties or discos five times or less per month during the last six months in Hong Kong and in Shenzhen respectively. Approximately 70% of the club drug users were accompanied by their friends to rave parties or discos in both Hong Kong and Shenzhen. Approximately 30% of them were accompanied by boy/girlfriends and around 20% by classmates for both places (Fig. 18).

Figure 19 shows the reasons for attending rave parties or discos were mostly 'to have uninhibited fun', 'to relax', 'to seek new exciting experiences' and 'to find new boy/girlfriend' for both Hong Kong and Shenzhen. However, a marked difference was shown for the reason 'to buy cheaper drugs' between individuals who go to Hong Kong and those who go to Shenzhen. It was shown that 41.2% of club drug users gave this reason for going to Shenzhen as oppose to 15% given by users going to rave parties or discos in Hong Kong (Fig. 19).

The source in which the club drugs were obtained in Hong Kong or in Shenzhen were mainly from friends (83.6% for HK and 78.9% for Shenzhen) followed by triad societies (45.5% for HK and 35.2% for Shenzhen) and drugs dealers at discos or rave parties (49.2% for HK and 57.3% for Shenzhen) (Fig. 20)

Apart from rave parties or discos, friend's house (54.1%), park (48.6%), 'at home' (35.8%) and street corner (33.3%) were the venues in which club drugs were usually

taken in Hong Kong. In Shenzhen, parks (27.6%) followed by friend's house (24.1%) were the places where club drugs users usually take their drugs (Fig. 21).

Table 5. Prevalence of drug uses in cross–border and non cross-border drug users in Hong Kong and Shenzhen

Prevalence of each club drug use in Hong Kong in the periods of lifetime drug use, drug use in the last 12 months and drug use in the last 30 days. Pattern of drug use between the cross-border and non cross-border drug users were compared. The cross-border drug users were further divided into i) pattern of drug use in Hong Kong and ii) pattern of drug use in Shenzhen. Significant difference was calculated using Yate's χ^2 analysis between cross-border drug users and non cross-border drug users in a specific period of drug use in Hong Kong as well as between the Hong Kong and Shenzhen cross-border group; $p<0.05$ was regarded as statistically significant.

Table 5. Pattern of drug use amongst cross-border and non cross-border users in Hong Kong and in Shenzhen

	Lifetime (%)			Last 12 Months (%)			Last 30 days (%)		
	Cross-border drug users (Pattern of drug use in Hong Kong) (N=199)	**Cross-border drug users** (Pattern of drug use in Shenzhen) (N=199)	**Non-cross border drug users** (Pattern of drugs use in Hong Kong) (N=154)	**Cross-border drug users** (Pattern of drugs use in Hong Kong) (N=199)	**Cross-border drug users** (Pattern of drugs use in Shenzhen) (N=199)	**Non-cross border drug users** (Pattern of drugs use in Hong Kong) (N=154)	**Cross-border drug users** (Pattern of drugs use in Hong Kong) (N=199)	**Cross-border drug users** (Pattern of drugs use in Shenzhen) (N=199)	**Non-cross border drug users** (Pattern of drugs use in Hong Kong) (N=154)
Ketamine	88.4	91.0	89.0	81.9	84.1	81.2	55.3#	39.7	53.9
Ecstasy	83.4	87.9	75.3	76.9*	78.4	60.4	42.2	40.2	34.4
Marijuana	87.9* #	78.9	79.2	82.4#	68.7	74.0	48.5#	28.3	39.0
Ice	31.7 #	9.7	28.6	18.1#	9.1	20.1	7.0	2.0	11.0
Alcohol	86.9	85.9	79.2	82.9	80.1	77.9	68.3#	39.9	63.0
Cigarette	87.4	88.4	81.8	83.9	80.1	81.2	70.2#	43.9	72.7
Benzodiazepines	31.2* #	15.6	14.9	18.6*	11.4	9.1	4.5	5.1	3.2
Cough Mixture	24.1#	9.0	17.5	17.2	10.2	12.3	8.1	4.0	1.3
Solvents	10.6#	1.5	9.7	2.5	2.9	4.5	1.0	2.0	3.9

* Significant difference found between cross-border and non cross-border drug users in a specific period of drug use in Hong Kong

Significant difference found in drug use in a specific period between Hong Kong and Shenzhen of the same group cross-border club drug users

Fig. 17) Places outside of Hong Kong in which club drugs were used among 366 club drugs users.

Fig. 18) The kinds of people that accompany the club drug users to the raves parties or discos in Hong Kong/ in Shenzhen during the last 6 months.

Fig. 17 Places outside of Hong Kong in which club drugs were used.

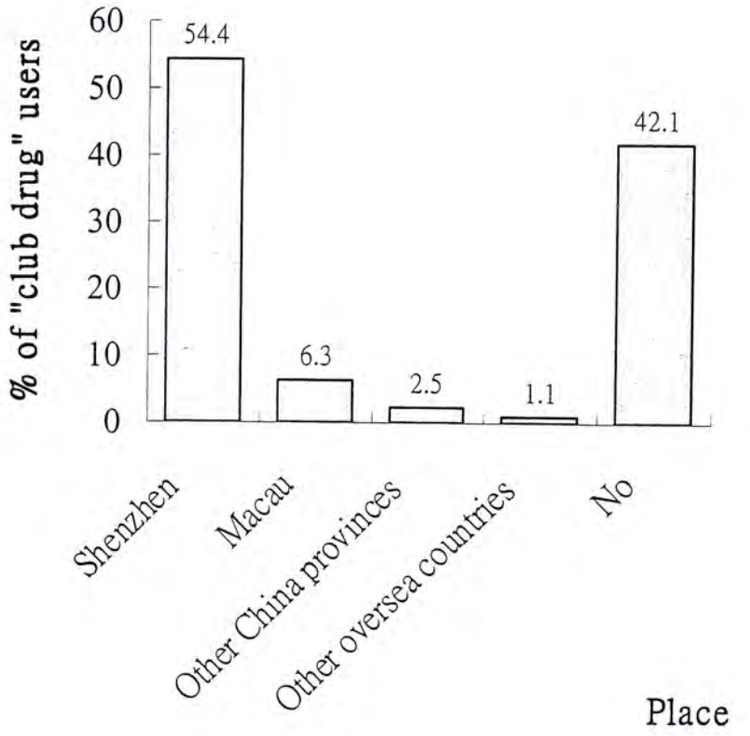


Fig. 18 The kinds of people that accompany the club drug users to the raves parties or discos in Hong Kong/ in Shenzhen during the last 6 months

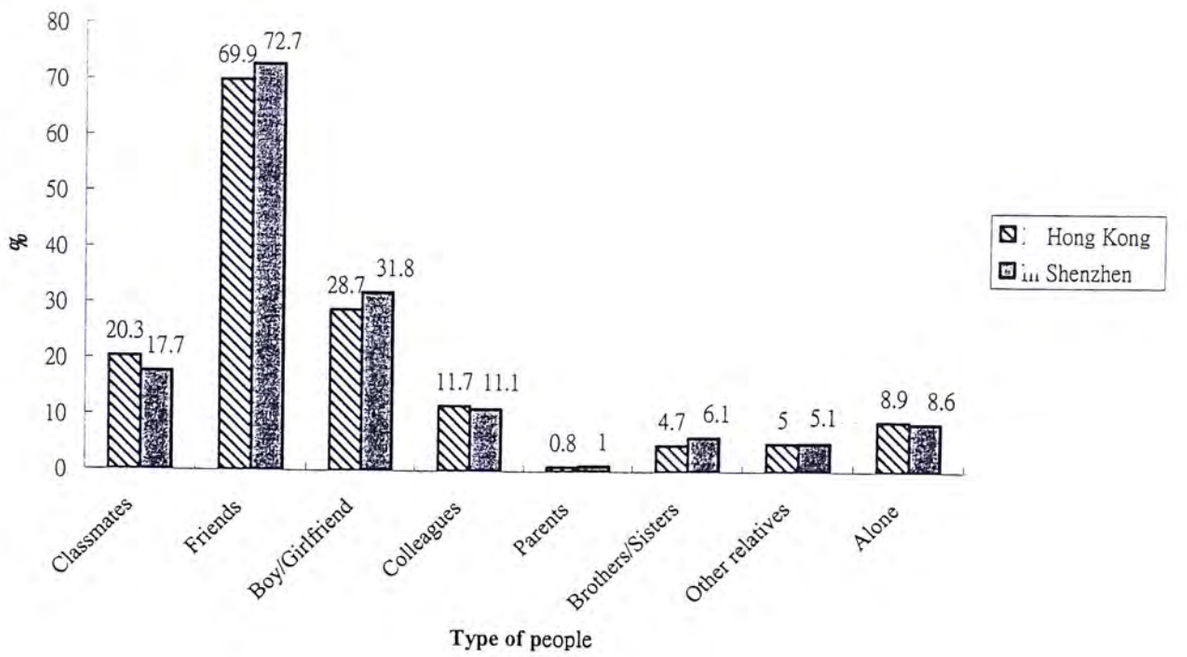


Fig. 19) Reasons for attending rave parties or discos in Hong Kong/ in Shenzhen.

Fig. 20) Source of drugs used in Hong Kong/ in Shenzhen.

Fig. 19 Reasons for attending rave parties or discos

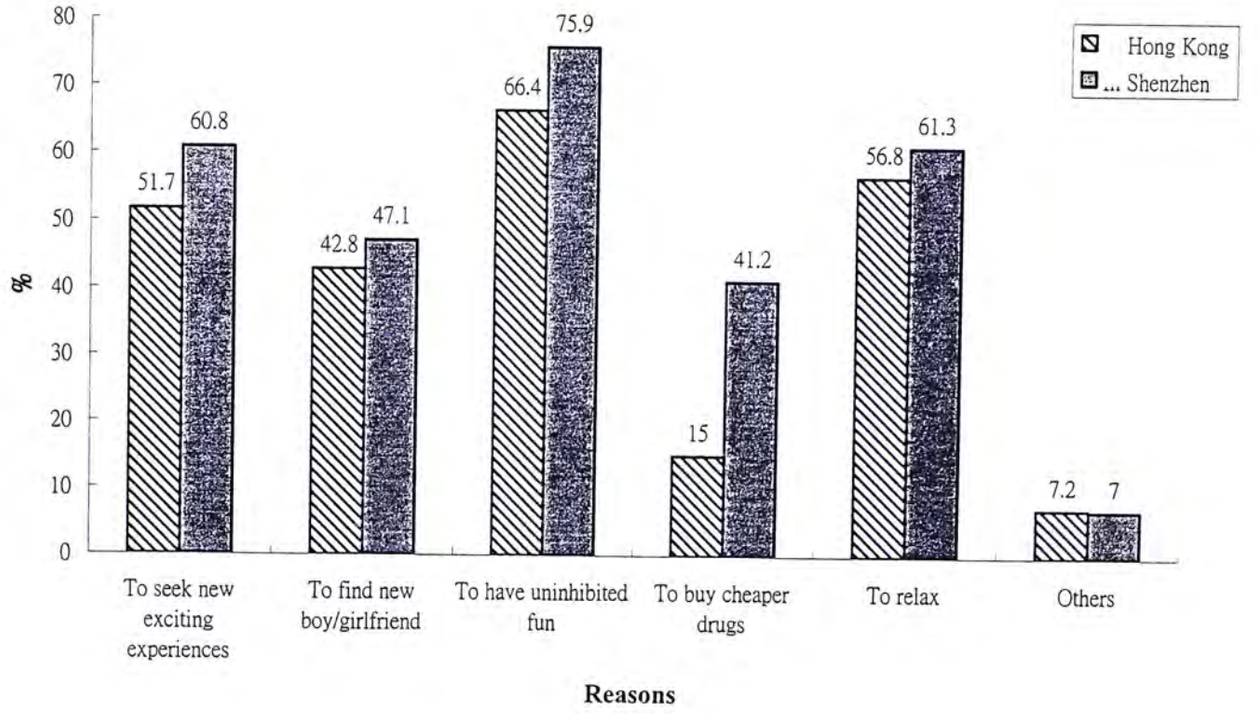


Fig. 20 Source of drugs used

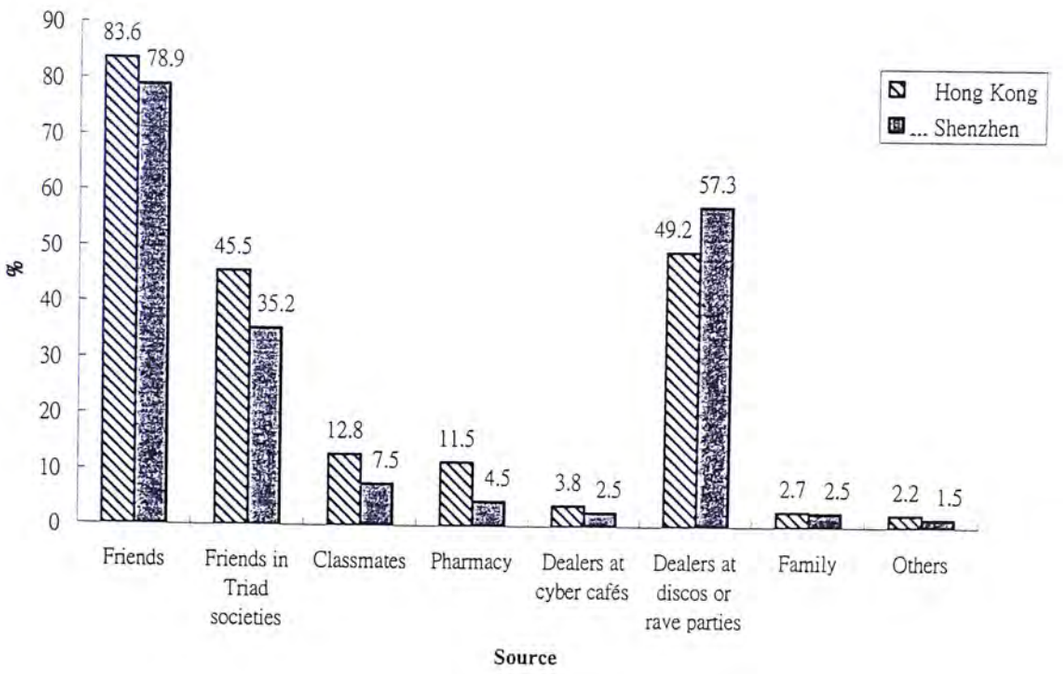
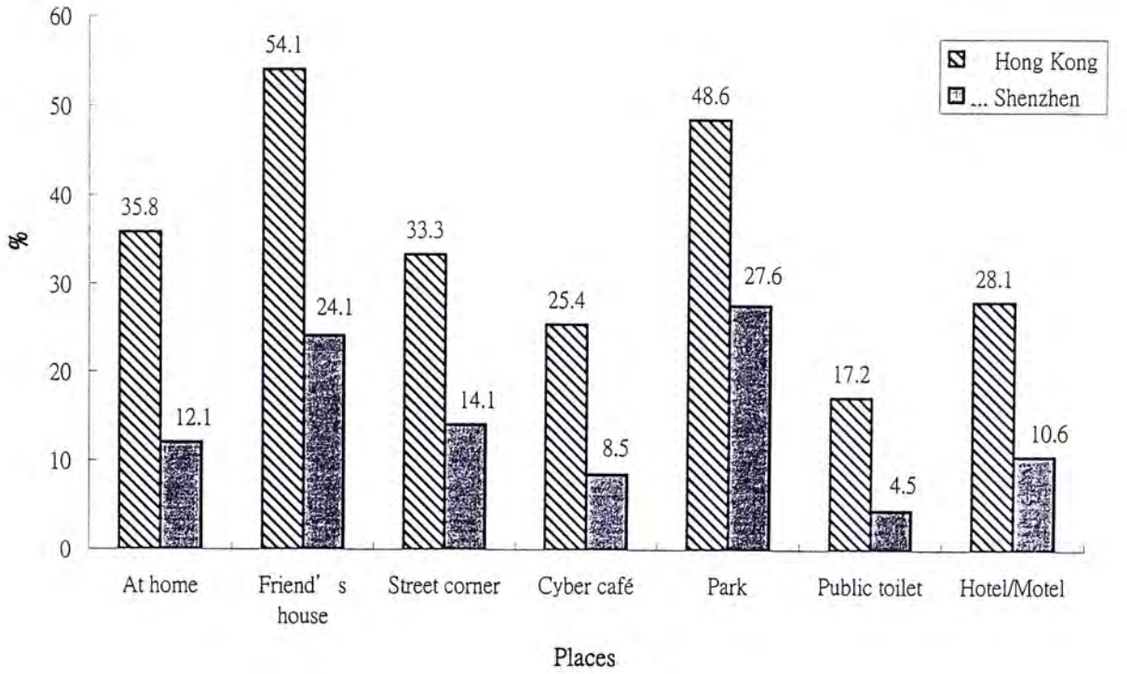


Fig. 21) Other venues in which drugs were taken in Hong Kong/ in Shenzhen.

Fig. 21 Other venues in which drugs were taken



3.1.9 Cause of drug use

Table 6 summarizes the most popular functions according to each club drug use with the top five percentages marked. Moreover, the age and gender of the club drug users in each club drug use were also reported. The functions were classified according to Boys A. et al. (2001) to reflect the effects of club drug use in the past year.

Ketamine use (n = 201)

The mean age of 'the most frequent use of ketamine' users were 17.9±2.5 (range in 13-29) with 67.8% males and 32.2 % females. Overall the most popular functions for ketamine use were to "ENJOY COMPANY" (80.1%), "ELATED/EUPHORIC" (68.3%), "RELAX" (67.3%), "KEEP GOING" (60.9%) and "FEEL BETTER" (54.0%). Seven of the 18 function items were endorsed by over half of those who had used ketamine on more than one occasion in the past year. By looking at the results we can predict that ketamine use mainly interferes with the two domains, they were "changing mood" and "social purpose".

'Ecstasy' use (n = 78)

The mean age of 'the most frequent use of 'ecstasy'' users were 18.7±2.3 (range in 14-24) with 73.1% males and 26.9% females. Overall the most popular functions for 'ecstasy' use were to "ENJOY COMPANY" (83.3%), "ELATED/ EUPHORIC" (75.6%), "KEEP GOING" (71.8%), "FEEL BETTER" (54.0%) and "STOP WORRYING" (43.6%). Seven of the 18 function items were endorsed by over half of those who had used 'ecstasy' on more than one occasion in the past year. By looking at the results we can predict that 'ecstasy' use mainly interferes with the two domains,

they were "changing mood" and "social purpose".

Marijuana use (n = 53)

The mean age of 'the most frequent use of marijuana' users were 17.7±2.7 (range in 14-27) with 86.8% males and 13.2 % females. Overall the most popular functions for marijuana use were to "ENJOY COMPANY" (71.7%), "ELATED/ EUPHORIC" (69.8%), "RELAX" (60.4%), "KEEP GOING" (50.9%) and "SLEEP" (43.4%). Four of the 18 function items were endorsed by over half of those who had used marijuana on more than one occasion in the past year. By looking at the results we can predict that marijuana use mainly interferes with the three domains including "changing mood", "physical effects" and "social purpose".

'Ice' use (n = 13)

The mean age of 'the most frequent use of 'ice'' users were 19.0±2.1 (range in 17-23) with 69.2% males and 30.8 % females. Overall the most popular functions for marijuana use were to "STAY AWAKE" (84.6%), "WORK" (76.9%), "ENJOY COMPANY" (69.2%), "INCREASE CONFIDENCE" (61.5%) and "KEEP GOING" (61.5%). Six of the 18 function items were endorsed by over half of those who had used 'ice' on more than one occasion in the past year. By looking at the results we can predict that 'ice' use mainly interferes with the two domains including "physical effects" and "social purpose".

In general, "social purpose" domain was the common function of substance use found in all the above club drugs. Other domains like "changing mood" and "physical effects" have also shown to be related to club drug use. In general, the prevalence of

'the most frequent drug use' by age in the order from the youngest to the oldest, they were marijuana, ketamine, 'ecstasy' and 'ice'; the prevalence of 'the most frequent drug use' by gender were in the order from the most prevalence for males to be marijuana, 'ecstasy', ketamine, and 'ice'.

3.1.10 The negative effects of drug use

The negative effects of physical and behavioural symptoms shown within 24 hours after taking drugs were investigated base on the specific club drug that the club drug users most frequently used. The results were categorized by the most popular drug used out of 4 most popular drugs reported to be used, namely, ketamine, 'ecstasy', marijuana and 'ice' (Table 7).

202 ketamine users reported to have physical symptoms like 'decrease in appetite' (71.3%), 'poor physical coordination' (55.0%) and 'extreme hyperactivity, e.g. dancing' (51.5%) (Table 7). Behavioural symptoms reported include 'feeling depressed or uninterested in things more than 24 hours' (58.7%), 'hallucination' (54%), and 'amnesia' (51.5%) (Table 8).

78 'ecstasy' users reported physical symptoms like 'loss of appetite' (71.8%), 'inability to sleep' (69.2%) and 'poor physical coordination' (66.2%) within 24 hours after taking 'ecstasy' (Table 7). Behavioural symptoms reported include 'hallucination' (61.5%), 'negatively affected their job or school performance' (59%) and 'amnesia' (47.4%) (Table 8).

53 marijuana users reported to have physical symptoms like 'decrease in appetite'

(56.6%), 'dizzy; headache' (49.1%), and 'irregular heartbeat' (34%) (Table 7). Other behavioural symptoms like 'hallucination' (43.4%), 'amnesia' (39.6%) and 'negatively affected their job or school performance' (59%) were also found (Table 8).

13 'ice' users had the physical symptoms like 'decrease in appetite', (76.9%), 'inability to sleep' and 'sweaty palm; shaking hands' (Table 7). 84.6% frequent 'ice' users had ' hallucinations', 76.9% had 'feeling suspicious or paranoid of people for more than 24 hours' and 'jumpy or nervousness for more than 24 hours' (Table 8).

Table. 6) Proportion (%) of those who have used [substance] more than once, who endorsed each functional statement for their use in the past year.

Roman numerals have been used to indicate the functions with the top five average scores noted by 'i to v' with 'i' shown the highest score. It also shows means for the total number of different items endorsed by individual users and the internal reliability of the function scales for each substance using Chronbach's α coefficients.

Table 6

Used [substance] to…	Ketamine (n = 201)	Ecstasy (n = 78)	Marijuana (n = 53)	Ice (n = 13)
Make yourself feel better when down or depressed (FEEL BETTER)	54.0[v]	53.8[v]	43.4	38.5
Help you stop worrying about a problem (STOP WORRYING)	44.6	43.6	39.6	30.8
Help you relax (RELAX)	67.3[iii]	71.8[iv]	60.4[iii]	53.8
Help you feel elated or euphoric (ELATED/ EUPHORIC)	68.3[ii]	75.6[ii]	69.8[ii]	53.8
Just get really stoned or intoxicated (INTOXICTED)	24.3	29.5	17.0	30.8
Enhance feelings when having sex (ENHANCE SEX)	10.9	17.9	22.6	30.8
Help you to stay awake (STAY AWAKE)	32.3	46.2	26.4	84.6[i]
Help you lose weight (LOSE WEIGHT)	21.8	26.9	17.0	46.2
Help you to sleep (SLEE)	31.2	19.2	45.2[v]	38.5
Help you enjoy the company of your friends (ENJOY COMPANY)	80.1[i]	83.3[i]	71.7[i]	69.2[iii]
Help you feel more confident or more able to talk to people in a social situation (INCREASE CONFIDENCE)	32.7	37.2	26.4	61.5[iv]
Help you lose your inhibitions (LOSE INHIBITIONS)	46.5	50.0	34.0	30.8
Help you keep going on a night out with friends (KEEP GOING)	60.9[iv]	73.1[iii]	50.9[iv]	61.5[v]
Help you to concentrate or to work or study (WORK)	3.0	3.8	7.1	76.9[ii]
Enhance an activity such as listening to music or playing a game or sport (ENHANCE ACTIVITY)	52.0	52.6	42.9	53.8
Help make something you were doing less boring (DECREASE BOREDOM)	52.5	42.3	41.1	30.8
Improve the effects of other substances (IMPROVE EFFECTS)	18.3	16.9	23.2	7.7
Help ease the after effects of other substances (AFTER EFFECTS)	6.0	3.8	8.9	30.8
Total number of items in the scale	18	18	18	18
Chronbach's α for scale items	0.77	0.73	0.83	0.91
Mean total number of different functions endorsed for use of [substance] (range)	6.9 (0-16)	7.3 (0-14)	6.3 (0-15)	8.2 (0-17)

Table. 7 Physical symptoms shown within 24 hours after taking drugs

	% of the club drug users			
Physical symptoms	Ketamine (202)	Ecstasy (78)	Marijuana (53)	Ice (13)
Decrease in appetite	71.3	71.8	56.6	100.0
Poor physical coordination	55.0	66.2	24.5	61.5
Inability to sleep	43.1	69.2	35.8	76.9
Sweaty palms; shaking hands	47.5	52.6	26.4	76.9
Dizzy; headache	45.0	42.3	49.1	53.8
Irregular heartbeat	38.1	38.5	37.0	69.2
Nausea, vomiting	50.5	46.2	32.7	69.2
Extreme hyperactivity, (e.g. dancing)	51.5	53.8	26.4	69.2

Table. 8 Behavioral symptoms shown within 24 hours after taking drugs

	% of the 'club drug'users			
Behavioural symptoms	Ketamine(202)	Ecstasy (78)	Marijuana (53)	Ice (13)
Amnesia	51.5	47.4	39.6	53.8
Hallucination	54.0	61.5	43.4	84.6
Feeling depressed or uninterested in things for more than 24 hours	58.7	34.6	18.9	61.5
Feeling suspicious or paranoid of people for more than 24 hours	23.0	23.1	32.1	76.9
Negatively affected your family relationships and friendships	22.8	21.8	25.0	69.2
Negatively affected your job or school performance	45.5	59.0	34.0	69.2
Jumpy or nervous for more than 24 hours	22.9	24.4	17.0	76.9
Twitch	0.5	1.3	5.7	0
Become violent	23.3	19.2	20.8	46.2
Suicide attempt	4.0	6.4	9.4	23.1

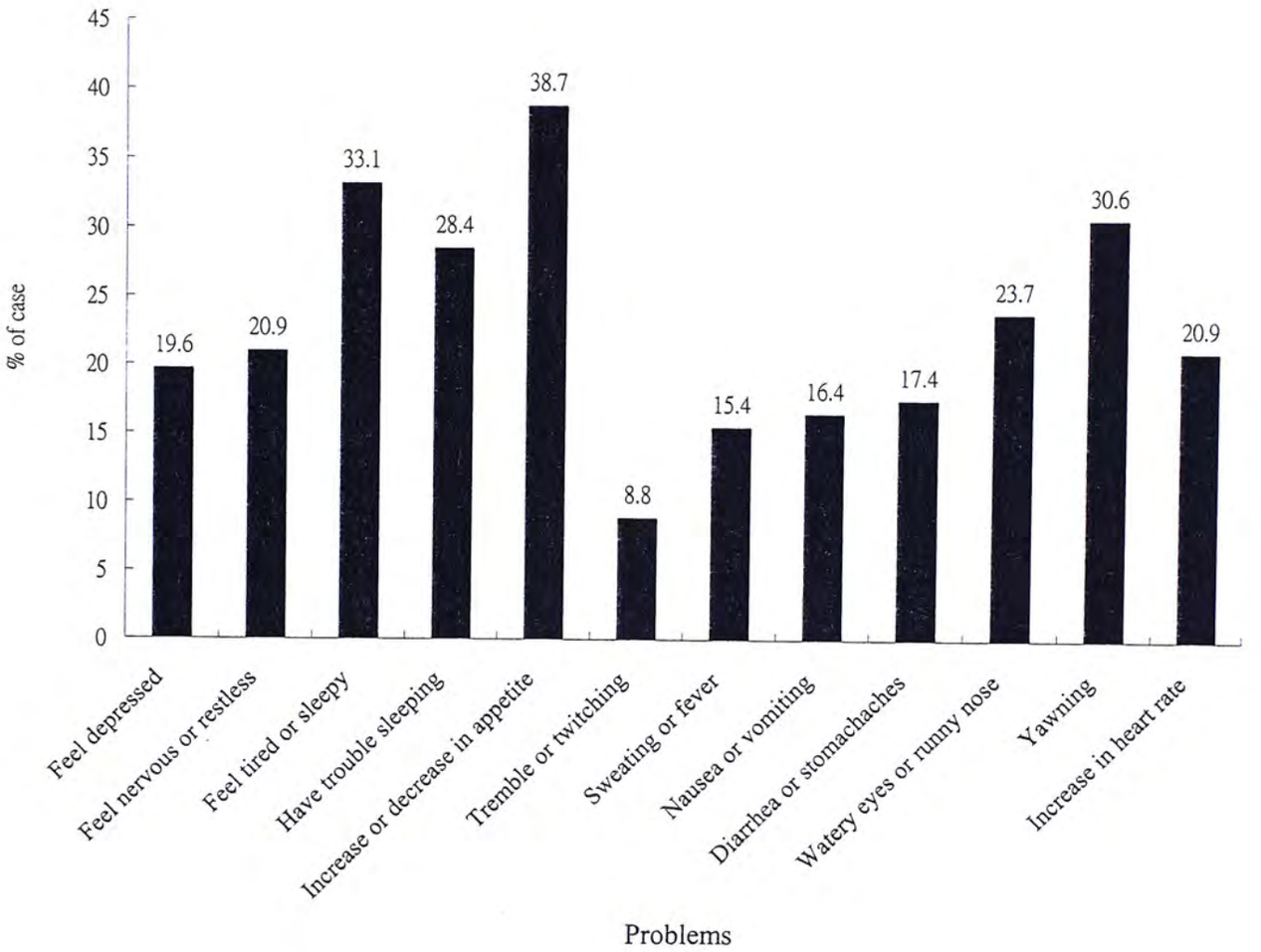
3.1.11. Potential tolerance/ dependence

After having stopped, cut down or quit taking drugs, 38.7% of club drug users found an 'increase in appetite', 33.1% 'feel tired or sleepy', 30.6% developed 'yawning' symptoms, 28.4% 'have trouble sleeping' and 23.7% have 'watery eyes or runny nose' (Fig. 22).

27.2% club drugs users spent a great deal of time in those activities necessary to obtain the drugs or use the drugs, e.g. in a period of a month or more. 72.8% subjects have ever wanted to or tried to cut down on drugs and 27.7% subjects found they could not stop or cut down using drugs. 78% claimed that they needed to increase the quantity of drugs by 50% or more and 48.5% needed markedly increased amounts of drugs to get an effect or found that they could no longer get high on the amount they used to use. 40.7% have used drugs for more days or in larger amounts than before. 38% club drug users have given up or greatly reduced important activities with friends or relatives or at work in order to use drugs. 52.9% subjects have received objections from family, friends, boss or people at work or school because of drug use of which 74.2% continue to use drugs after they realized it was causing a problem. 26.9% club drug users have been under the effects of drug in a situation where it increased their chances of getting hurt, for instance, when driving, using knives or machinery, crossing against traffic, climbing or swimming (Fig. 22).

Fig. 22) Problems caused after having stopped, cut down or quit taking drugs among 366 club drugs users.

Fig. 22 Problems caused after having stopped, cut down or quit taking drugs



3.1.12 Knowledge about drugs of abuse

Seven questions regarding knowledge of drug abuse were asked and the summary of the respondents listed in Table 8. Generally the knowledge of the club drug users was quite good. Four out of 7 questions about harmfulness from taking club drugs were answered correctly by over 70% of the club drugs users on questions like 'can harm to your body', 'may be contaminated with other drugs', 'can cause permanent damage to your body' and 'using of needles to inject club drugs can cause AIDS'. 20.9% and 31.3% club drugs users did not believe that club drugs could cause mental problems and that they are addictive and may lead to dependence respectively (Table 9).

3.1.13 Psychological well-being

91.2% club drugs users felt stressful in their daily life of which 17% felt extremely pressurized. Only 8.8% of them felt no pressure at all. 52.1 % subjects were satisfied with their life while 29.5% were not happy about their life. 13.7% were even not sure whether they were happy about their present life style or not.

Table. 9) Knowledge about drugs of abuse among 366 club drugs uses.

Table 9 Knowledge about drugs of abuse

Questions of knowledge about drugs of abuse	%		
	Yes	No	I don' know
Do you think that the 'club drugs' can cause harm to your body?	83.8	6.0	10.2
Do you think that the 'club drugs' can cause mental problems?	67.3	20.9	11.8
Do you think that the 'club drugs' are addictive and may lead to dependence?	58.2	31.3	10.4
Do you think some of the drugs that you take may be contaminated with other drugs?	79.7	6.3	14.0
Do you think that the 'club drugs' can cause permanent damage to your body?	70.1	11.0	19.0
Do you think that the taking of the 'club drugs' can result in death?	64.8	17.3	17.9
Do you think the using of needles to inject 'club drugs' can cause AIDS?	78.8	5.2	15.9

3.2 Personality trait assessments

3.2.1 Personality traits between club drugs users and controls

When 360 club drug users were compared to 303 controls in the personality traits studied, in general club drugs users had higher scores for the SSS-V scale. For the BIS/BAS scales, club drug users also score higher for 'BAS Reward responsiveness', 'BAS fun seeking' and 'BAS drive'. However, a lower score for the 'BIS' in BIS/BAS scale in the club drug users were also observed when compared to controls (Table 10). Except for 'BAS Reward responsiveness' in BIS/BAS scale, a significant difference was shown in the score of 'boredom susceptibility' ($p<0.0001$, $|0.68< CI<1.23|$), 'disinhibition' ($p<0.0001$, $|3.39< CI < 4.04|$), 'experience seeking' ($p<0.0001$, $|1.69< CI < 2.25|$), 'thrill and adventure seeking' ($p=0.008$, $|0.15< CI < 0.99|$) of the SSS-V scale; 'BIS' ($p<0.0001$, $|-1.56< CI < -0.58|$), 'BAS fun seeking' ($p<0.0001$, $|0.98< CI < 1.59|$), and 'BAS drive' ($p<0.0001$, $|0.52< CI < 1.16|$) in BIS/BAS scale between club drugs users and controls (Table 10).

3.2.2 Personality trait by gender

By comparing 263 male club drug users to 193 male controls in personality traits, male club drug users had significantly higher scores for 'boredom susceptibility' ($p<0.0001$, $|0.62< CI<0.1.33|$) 'disinhibition' ($p<0.0001$, $|3.20< CI < 4.06|$), 'experience seeking' ($p<0.0001$, $|1.55< CI < 2.29|$), 'thrill and adventure seeking' ($p=0.005$, $|0.27< CI < 1.30|$) in the SSS-V scale; 'BAS drive' ($p<0.0001$, $|0.44< CI < 1.24|$), 'BAS fun seeking' ($p<0.0001$, $|0.83< CI < 1.61|$) but lower scores for

'BIS' ($p<0.0001$, $|-1.41< CI < -0.11|$) in the BIS/BAS scales (Table 11). Although drug users also scored higher in the 'BAS Reward responsiveness' in BIS/BAS scale, there is no significant difference between cases and controls.

When 97 female club drug users were compared to 110 female controls in personality traits, female club drug users had significantly higher scores for 'boredom susceptibility' ($p=0.001$, $|0.38< CI<1.42|$) 'disinhibition' ($p<0.0001$, $|3.12< CI < 4.24|$), 'experience seeking' ($p<0.0001$, $|1.52< CI < 2.51|$) in the SSS-V scale; 'BAS drive' ($p=0.005$, $|0.29< CI < 1.56|$), 'BAS fun seeking' ($p<0.0001$, $|0.88< CI < 2.05|$) but lower scores for 'BIS' ($p<0.0001$, $|-2.01< CI < -0.25|$) in the BIS/BAS scales (Table 12). Females also scored higher in 'thrill and adventure seeking' in the SSS-V scales and 'BAS Reward responsiveness' in the BIS/BAS scale, there is however no significant difference between cases and controls.

Significantly higher scores in 'boredom susceptibility' ($p=0.018$, $|0.09< CI < 1.06|$), 'disinhibition' ($p<0.0001$, $|3.12< CI < 4.24|$), 'thrill and adventure seeking' ($p<0.0001$, $|-3.12< CI < -4.24|$) in the SSS-V scales and a lower score in 'BIS' ($p<0.025$, $|3.12< CI < 4.24|$) in the BIS/BAS scales were found when 263 male club drug users were compared to 97 female club drug users. While significantly higher scores in 'boredom susceptibility' ($p=0.014$, $|0.09< CI < 1.06|$), 'disinhibition' ($p<0.0001$, $|0.60< CI < 1.62|$) in the SSS-V scales and a lower score in 'BIS' ($p<0.0001$, $|-2.06< CI < -0.62|$) in the BIS/BAS scales were found when 193 male controls were compared to 110 female controls (Table 13).

3.2.3 Reliability

The coefficient Cronbach's alpha (α) of each subscale was calculated to assess the reliability of each subscale. This is particularly pertinent for the BIS/BAS subscales since this it is the first Chinese translation of such this test. The Cronbach's α were shown to be: 0.43 for 'boredom susceptibility', 0.77 for 'disinhibition', 0.48 for 'experience seeking' 0.77 for 'thrill and adventure seeking' in the SSS-V scales. For the BIS/BAS scales Cronbach's α were shown to be: 0.71 for 'BIS', 0.62 for 'BAS Reward responsiveness', 0.58 for 'BAS fun seeking' and 0.66 for 'BAS drive'. (Table 10).

Table 10.

Independent-samples t-test comparing the scores between 303 controls and 360 club drugs users in the subscales of SSS-V and BIS/BAS personality scales. Cronbach's α of the reliability of each subscale was shown.

Table 11.

Independent-samples t-test comparing the scores between 193 male control and 263 male club drug users in the subscales of SSS-V and BIS/BAS personality scales.

Table 10

	(Mean score ± SD)			
	Controls (n=303)	Club drug users (n=360)	T	α
Boredom susceptibility	2.5 ± 1.7	3.5 ± 1.9	6.8**	0.43
Disinhibition	2.8 ± 2.2	6.5 ± 2.0	22.4**	0.77
Experience seeking	3.4 ± 1.9	5.3 ± 1.8	13.9**	0.48
Thrill and adventure seeking	5.5 ± 2.9	6.1 ± 2.7	2.6*	0.77
BIS	20.2 ± 3.1	19.1 ± 3.3	-4.3**	0.71
BAS reward responsiveness	16.5 ± 2.0	16.5 ± 2.2	-0.071	0.62
BAS fun seeking	11.4 ± 2.2	12.7 ± 2.0	8.4**	0.58
BAS drive	11.9 ± 2.2	12.8 ± 2.2	5.2**	0.66

***p<0.05,**p<0.0001**

Table 11

	(Mean score ± SD)		
	Male controls (n=193)	Male club drug users (n=263)	T
Boredom susceptibility	2.7 ± 1.7	3.6 ± 1.8	5.5**
Disinhibition	3.2 ± 2.4	6.8 ± 1.9	17.4**
Experience seeking	3.5 ± 2.0	5.4 ± 1.8	11.1**
Thrill and adventure seeking	5.7 ± 2.8	6.5 ± 2.5	3.4**
BIS	19.7 ± 3.0	18.9 ± 3.4	-2.6**
BAS reward responsiveness	16.4 ± 2.1	16.5 ± 2.2	0.3
BAS fun seeking	11.5 ± 2.0	12.7 ± 2.0	6.7**
BAS drive	12.1 ± 2.0	12.9 ± 2.1	4.2**

***p<0.05, **p<0.0001**

Table 12.

Independent-samples t-test comparing the scores between 110 female controls and 97 female club drug users in the subscales of SSS-V and BIS/BAS personality scales.

Table 13.

Independent-samples t-test comparing the scores between males and females within groups (control and club drug users, respectively) in the subscales of SSS-V and BIS/BAS personality scales.

Table 12

	(Mean score ± SD)		
	Female controls (n=110)	Female club drug users (n=97)	T
Boredom susceptibility	2.2 ± 1.7	3.0 ± 2.0	3.3*
Disinhibition	2.1 ± 1.8	5.8 ± 2.2	13.3**
Experience seeking	3.2 ± 1.8	5.1 ± 1.8	8.0**
Thrill and adventure seeking	5.2 ± 3.1	4.9 ± 2.8	-0.8
BIS	21.0 ± 3.3	19.7 ± 3.0	-3.0*
BAS reward responsiveness	16.7 ± 2.0	16.6 ± 2.3	-0.4
BAS fun seeking	11.2 ± 2.1	12.6 ± 2.0	4.8**
BAS drive	11.7 ± 2.0	12.4 ± 2.4	2.5*

***p<0.05, **p<0.0001**

Table 13

	Control			Club drugs		
	(Mean score ± SD)			(Mean score ± SD)		
	Male (n=193)	Female (n=110)	T	Male (n=263)	Female (n=97)	T
Boredom susceptibility	2.7±1.7	2.2±1.7	2.5#	3.6±1.8	3.0±2.0	2.7*
Disinhibition	3.2±2.4	2.1±1.8	4.6##	6.8±1.9	5.8±2.2	3.9**
Experience seeking	3.5±2.0	3.2±1.8	1.3	5.4±1.8	5.1±1.8	1.3
Thrill and adventure seeking	5.7±2.8	5.2±3.1	1.3	6.5±2.5	4.9±2.8	5.3**
BIS	19.7±2.9	21.0±3.3	-3.6##	18.9±3.4	19.7±3.0	-2.1*
BAS reward responsiveness	16.4±2.1	16.7±2.0	-1.3	16.5±2.2	16.6±2.3	-0.5
BAS fun seeking	11.5±1.9	11.2±2.1	1.2	12.7±2.0	12.6±2.0	0.6
BAS drive	12.1±2.0	11.7±2.0	1.7	12.9±2.1	12.4±2.4	1.6

 # p<0.05, ##p<0.0001 – significant difference between male and female controls

*** p<0.05, **p<0.0001 – significant difference between male and female club drug users**

3.3 Genotyping

3.3.1 G1947A,Val108/158 Met polymorphism in the catechol-*O*-methyl-transferase (COMT) gene

The 130bp PCR product showed that the homozygous GG for high activity COMT was represented as a single 114 bp band, while homozygous AA for low activity COMT was represented by the presence of a 96 bp band. Both the 96 bp and 114 bp bands were found in the heterozygotes AG (Fig.23).

Table 14a shows the genotype distributions and allele frequencies of G and A of the G1947A, $Val^{108/158}$ Met polymorphism of the COMT gene in club drugs users and controls. It reveals that 154 controls (51%) carried the GG homozygous genotype, 15 controls (5%) carried the AA homozygous genotype and 134 (44%) were carriers of the GA heterozygote genotype. In the club drugs users, it was found that there were 177(64%) and 15(5%) with GG and AA homozygous genotype respectively. 85 subjects (31%) were GA heterozygous. Yates χ^2 analysis showed that $\chi^2_{(2)}=11.419$; p=0.003. The G allele was the most common allele in both controls (73%) and club drugs users (79%). The prevalence of the G allele in the club drugs users was significantly higher ($\chi^2_{(1)}= 6.298$; p=0.013.) than that of the control group, while a higher prevalence of the A allele was found in controls (27%) than in the club drugs users (21%). It can be noted that the higher frequency of G allele in the control group contributed to the major genotype homozygous GG (n=154, 51%) and heterozygous GA (n=134, 44%). In the club drug users, the number of homozygous GG individuals was two fold that of GA heterozygotes (177 vs 85). The relatively high percentage of homozygous GG subjects in the club drug users when compared to the controls

contributed to the high G allele frequency (64% in club drug users vs 51% in controls). The percentage of heterozygous GA individuals in the club drug users was lower than that of controls (31% in club drug users vs 44% in controls).

Table 14b represents the SSS-V and BIS/BAS scores for club drug users with the three different genotypes (AA, AG, GG). Significant association between COMT genotype and 'BAS drive' in the BIS/BAS scale ($p=0.03$) was shown with the interaction between genotype AG and GG ($p=0.027$; $|0.06>CI>1.43|$) when all three groups were compared (one-way ANOVA), subjects homozygous for the G allele displayed lower BAS drive scores. By comparing the grouping AG+AA vs GG, individuals with two copies of the G allele scored significantly lower than individuals with at least one of the A allele on the subscale 'boredom susceptibility' ($p=0.02$, $|0.069>CI>0.995|$) in the SSS-V scale and BAS drive ($p=0.01$, $|0.196>CI>1.251|$). When the genotypes were grouped together in AG + GG vs AA, to compare between subjects with a G allele versus those without a G allele, no significant associations between the COMT polymorphism and SSS-V as well as BIS/BAS scales were found.

Table 14c represents the SSS-V and BIS/BAS scores for the control group with the three different genotypes (AA, AG, GG). No significant association between COMT genotype and the two personality trait scales when all three groups were compared (one-way ANOVA). When the genotypes were grouped together in AG + AA vs GG, to compare between subjects with an A allele versus those without an A allele. Individuals with at least one of the A allele scored significantly lower than individuals with two copies of the G allele on the subscale 'BAS fun seeking' ($p=0.02$, $|-1.0>CI>-0.01|$). By comparing the grouping AG+GG vs AA, none of the significant

association was found.

Fig 23. The 130bp PCR product was subjected to Nla III digestion and the resulting fragments were resolved on a 4% agarose gel that was stained with ethidium bromide. A DNA marker (GeneRuler™ 50bp DNA Ladder) was loaded on the left. Homozygous GG is shown as a 114bp band, homozygous AA is shown as a 96 bp band, heterozygous is shown as 114bp and 96bp bands.

Table 14a. Genotype distribution and allele frequencies of the G1947A, COMT $Val^{108/158}$ Met polymorphism in club drugs users and controls. The absolute number of cases and the percentage of total (in brackets) are shown. Yates χ^2 analysis showed that $\chi^2 = 11.419$; df =2; p=0.003 in the distribution of genotype frequency and $\chi^2 = 6.298$; df= 1; p=0.013 in the allelic frequencies between club drugs users and controls.

Fig. 23

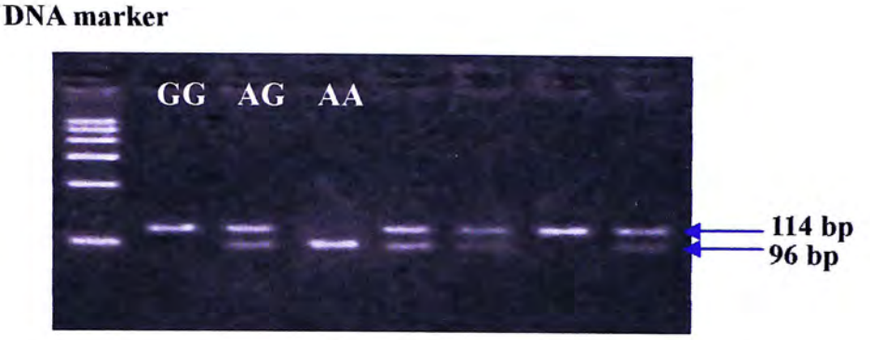


Table 14a.

Genotype distributions and allele frequencies of COMT

	Genotypes, n (frequencies)				Allele frequencies		
	A/A	A/G	G/G	(p)[1]	A	G	(p)
Club drugs users (277)	15(0.05)	85(0.31)	177(0.64)		115(0.21)	439(0.79)	
Normal control (303)	15(0.05)	134(0.44)	154(0.51)	0.003	164(0.27)	442(0.73)	0.013

[1] *P*-value of Yates χ^2 analysis

Table 14b

Scores of subscales of SSS-V and BIS/BAS Scales in club drug users with respect to COMT genotypes amongst 277 club drug users. The SSS-V and BIS/BAS scores for club drug users with the three different genotypes (AA, AG, GG) were shown. Comparisons between all three genotype groups with mean scores of SSS-V and BIS/BAS subscales were carried by one-way ANOVA. The mean scores of SSS-V and BIS/BAS subscales were compared between subsamples defined by the presence or absence of alleles using Student's t-test.

Table 14b Scores of subscales of SSS-V and BIS/BAS Scales in club drug users with respect to COMT genotypes.

COMT (club drug users)									
		SSS-V scores (mean±SD)[a]				BIS/BAS scores (mean±SD)[a]			
Genotype	N	Boredom susceptibility	Disinhibition	Experience seeking	Thrill and adventure seeking	BIS	BAS reward responsiveness	BAS fun seeking	BAS drive
A/A	15	3.5±1.4	6.8±1.9	5.1±2.2	5.9±2.8	20.7±3.2	16.2±2.1	12.5±2.1	13.1±2.2
A/G	85	3.9±1.9	6.7±2.0	5.4±1.9	6.1±2.7	19.0±3.3	16.8±2.0	13.0±2.1	13.3±2.2
G/G	177	3.3±1.9	6.4±2.0	5.3±1.6	5.9±2.7	19.2±3.3	16.4±2.2	12.6±1.9	12.5±2.1
A/G+A/A	100	3.8±1.8	6.7±1.9	5.4±1.9	6.1±2.7	19.2±3.3	16.7±2.0	12.9±2.1	13.3±2.2
A/G+G/G	262	3.5±1.9	6.5±2.1	5.3±1.7	6.0±2.7	19.2±3.3	16.5±2.1	12.7±2.0	12.8±2.2
P[b]		0.59	0.51	0.78	0.78	0.17	0.25	0.29	0.03
P[c]		0.02	0.26	0.70	0.51	0.98	0.19	0.18	0.01
P[d]		0.98	0.54	0.66	0.99	0.07	0.57	0.73	0.54

[a] Personality test scores are given as T-scores, which are standardized using normative data to have a mean ± SD.

[b] Significant levels of comparisons between all three genotype groups (one-way ANOVA)

[c] Significant levels of comparisons between A/G + A/A and G/G (t-test).

[d] Significant levels of comparisons between A/G + G/G and A/A (t-test).

Table 14c

Scores of subscales of SSS-V and BIS/BAS Scales in club drug users with respect to COMT genotypes amongst 303 controls. The SSS-V and BIS/BAS scores for club drug users with the three different genotypes (AA, AG, GG) were shown. Comparisons between all three genotype groups with mean scores of SSS-V and BIS/BAS subscales were carried by one-way ANOVA. The mean scores of SSS-V and BIS/BAS subscales were compared between subsamples defined by the presence or absence of alleles using Student's t-test.

Table 14c Scores of subscales of SSS-V and BIS/BAS Scales in controls grouped with respect to COMT genotypes.

		COMT (controls)							
		SSS-V scores (mean±SD)[a]				BIS/BAS scores (mean±SD)[a]			
Genotype	N	Boredom susceptibility	Disinhibition	Experience seeking	Thrill and adventure seeking	BIS	BAS reward responsiveness	BAS fun seeking	BAS drive
A/A	15	1.9±1.6	2.0±1.9	2.8±1.9	5.1±2.8	20.4±2.0	16.6±1.9	10.8±2.4	11.6±2.1
A/G	134	2.5±1.7	2.7±2.3	3.5±1.8	5.4±2.8	20.2±3.3	16.6±1.9	11.2±2.1	12.0±2.1
G/G	154	2.6±1.7	3.0±2.2	3.3±2.0	5.6±2.9	20.1±3.1	16.4±2.2	11.7±1.8	11.9±2.0
A/G+A/A	149	2.4±1.7	2.6±2.2	3.4±1.8	5.4±2.8	20.3±3.2	16.6±1.9	11.1±2.1	12.0±2.1
A/G+G/G	288	2.5±1.7	2.8±2.2	3.4±1.9	5.5±2.9	20.2±3.2	16.5±2.0	11.4±2.0	11.9±2.0
P[b]		0.34	0.22	0.42	0.74	0.91	0.49	0.06	0.67
P[c]		0.61	0.18	0.74	0.53	0.70	0.23	0.02\	0.62
P[d]		0.15	0.17	0.24	0.56	0.79	0.84	0.23	0.55

[a] Personality test scores are given as T-scores, which are standardized using normative data to have a mean ± SD.

[b] Significant levels of comparisons between all three genotype groups (one-way ANOVA)

[c] Significant levels of comparisons between A/G + A/A and G/G (t-test).

[d] Significant levels of comparisons between A/G + G/G and A/A (t-test).

3.3.2 T941G polymorphism in the monoamine oxidase A (MAO-A)

The 130bp PCR product showed that the homozygous TT was represented by the presence of a 130 bp single band, whereas genotype GG was represented by the presence of a 65 bp single band. Both the 65 bp and 130 bp bands were found in the heterozygote TG sample (Fig. 24)

Table 15a shows the genotype distributions and allele frequencies of G and T of T941G polymorphism in the MAO-A gene in male club drugs users. It reveals that 103 controls (54%) carried the GG homozygous genotype and 89 (46%) carried the TT homozygous genotype. Similarly, it was found that there were 103 (56%) and 81 (44%) club drugs users with GG and TT homozygous genotype respectively. Yates χ^2 analysis showed that $\chi^2_{(1)}=1.049$; p=0.679 (NS). Allelic frequency distribution shows that the G allele was the most common allele in both controls (54%) and subjects (55%). However, there was no significant different in allele frequencies between the control and subject groups (p=0.56) in the male club drug users.

Table 15b shows the genotype distributions and allele frequencies of G and T of T941G polymorphism in the MAO-A gene in female club drugs users. It reveals that 46 controls (42%) carried the GG homozygous genotype and 47 (43%) carried the TG heterozygous genotype and 17 (15%) were carriers of the TT genotype. Similarly, it was found that there were 37 (43%) club drugs users with TG heterozygous genotype. However, the GG and TT homozygous genotype was 25 (29%) and 24 (28%) respectively. Yates χ^2 analysis showed a marginal insignificant difference between controls and subjects ($\chi^2_{(2)}=5.74$; p=0.057). When the allelic frequencies were

examined, the T allele was observed in larger proportion of club drugs users than in controls (49% vs 37%). χ^2 test yielded a significant difference ($\chi^2_{(1)}$=6.278; p=0.012) between the two groups. The higher proportion of T was shown to be due to an increase in the number of TT homozygotes (28% in club drugs users vs 15% in controls). Moreover, the increase in TT homozygotes was accompanied by a decrease in GG homozygotes with no difference in the proportion of heterozygotes between the subjects and the controls (43%).

Table 15c shows the scores of the SSS-V and BIS/BAS subscales for the female club drug users when analyzed with the three genotypes (GG, TG, TT). Significant association between MAO-A genotype and BIS score in BIS/BAS scale (p=0.03) with an interaction between genotype GT and TT (p=0.041, |0.06>CI>3.56|) was found when compared to all three groups (one-way ANOVA), with subjects homozygous for the T allele displaying lower BIS scores. Individuals with two copies of the T allele scored significantly lower than individuals with at least one of the G allele (to compare those with TG+GG and those without a G allele) on BIS (p=0.008, |0.479>CI>3.078|). When the genotypes were grouped together in TG+TT vs GG to compare those with a T allele and those without a T allele, subjects homozygous for the G allele scored significantly higher than individuals with at least one of the T allele on BAS reward responsiveness (p=0.024, |-2.20>CI>-0.162|).

Table 15d shows the scores of the SSS-V and BIS/BAS subscales for the female control group when analysed with the three genotypes (GG, TG, TT). No significant association between MAO-A genotype and the personality trait subscales when compared to all three groups (one-way ANOVA). No significant difference on

personality traits was found when the genotypes were grouped together in TG+TT vs GG to compare with those with a T allele and those without a T allele or when the genotype was grouped in TG+GG vs TT to compare those with a G allele and those without a G allele.

Fig 24 The 130bp PCR product was subjected to Sat I digestion and the resulting fragments were resolved on a 4% agarose gel that was stained with ethidium bromide. A DNA marker (GeneRuler™ 50bp DNA Ladder) was loaded on the left. Homozygous TT is shown as a 130bp band, homozygous GG is shown as a 65 bp band, heterozygous TG is shown as both 65bp and 130bp bands.

Table 15a. Genotype distributions and allele frequencies of the MAO-A T941G polymorphism in male club drugs users and controls. The absolute number of cases and the percentage of total (in brackets) are shown. Yates χ^2 analysis showed that χ^2 =1.049; df=1; p=0.679 in the distribution of genotype frequency and χ^2 = 0.413; df = 1; p=0.56 in the allelic frequencies between club drugs users and controls.

Table 15b. Genotype distributions and allele frequencies of the MAO-A T941G polymorphism in female club drugs users and controls. The absolute number of cases and the percentage of total (in brackets) are shown. Yates χ^2 analysis showed that χ^2 =5.74; df=2; p=0.057 in the distribution of genotype frequency and χ^2 = 6.278; df = 1; p=0.012 in the allelic frequencies between club drugs users and controls.

Fig. 24

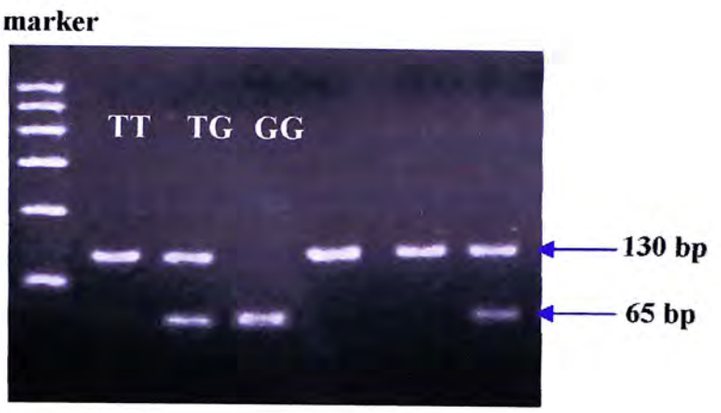


Table 15a.Genotype distributions and allele frequencies of MAO-A (Male)

	Genotypes, n (frequencies)				Allele frequencies		
	G/G	T/G	T/T	(p)[1]	G	T	(p)
Polydrug users (184)	103(0.56)	0(0)	81(0.44)		206(0.56)	162(0.44)	
Normal control (192)	103(0.54)	0(0)	89(0.46)	0.679	206(0.54)	178(0.46)	0.564

[1]*P*-value of Yates χ^2 analysis.

Table 15b Genotype distributions and allele frequencies of MAO-A (Female)

	Genotypes, n (frequencies)				Allele frequencies		
	G/G	T/G	T/T	(p)[1]	G	T	(p)
Polydrug users (86)	25(0.29)	37(0.43)	24(0.28)		87(0.51)	85(0.49)	
Normal control (110)	46(0.42)	47(0.43)	17(0.15)	0.057	139(0.63)	81(0.37)	0.012

[1]*P*-value of Yates χ^2 analysis.

Table 15c

Scores of subscales of SSS-V and BIS/BAS Scales in club drug users with respect to MAO-A genotypes amongst 86 female club drug users. The SSS-V and BIS/BAS scores for club drug users with the three different genotypes (GG, TG, TT) were shown. Comparisons between all three genotype groups with mean scores of SSS-V and BIS/BAS subscales were carried by one-way ANOVA. The mean scores of SSS-V and BIS/BAS subscales were compared between subsamples defined by the presence or absence of alleles using Student's t-test.

Table 15c Scores of SSS-V and BIS/BAS subscales in female club drug users with respect to MAO-A genotypes.

MAO-A (Female club drug users)

		SSS-V scores (mean±SD) [a]				BIS/BAS scores (mean±SD)			
Genotype	N	Boredom susceptibility	Disinhibition	Experience seeking	Thrill and adventure seeking	BIS	BAS reward responsiveness	BAS fun seeking	BAS drive
G/G	25	3.0±2.0	6.2±2.2	5.4±1.8	4.8±2.8	20.4±2.5	17.6±1.8	12.6±2.2	13.3±2.5
T/G	37	3.1±2.0	5.5±2.0	5.1±1.6	4.5±2.7	20.4±3.2	16.5±2.4	12.9±1.8	12.6±2.6
T/T	24	3.1±2.0	5.7±2.4	5.2±1.8	5.6±2.3	18.6±2.2	16.3±2.1	12.5±2.3	11.8±2.2
T/G+G/G	62	3.1±2.0	5.8±2.1	5.2±1.6	4.6±2.7	20.4±2.9	17.0±2.3	12.8±2.0	12.9±2.6
T/G+T/T	61	3.1±2.0	5.6±2.2	5.1±1.7	4.9±2.6	19.7±2.9	16.5±2.3	12.7±2.0	12.3±2.5
P [b]		0.99	0.54	0.73	0.27	0.03	0.07	0.73	0.13
P [c]		1.00	0.82	0.95	0.13	0.008	0.22	0.61	0.08
P [d]		0.90	0.28	0.45	0.92	0.34	0.02	0.72	0.10

[a] Personality test scores are given as T-scores, which are standardized using normative data to have a mean ± SD.

[b] Significant levels of comparisons between all three genotype groups (one-way ANOVA)

[c] Significant levels of comparisons between T/G+G/G and T/T (t-test).

[d] Significant levels of comparisons between T/G+T/T and G/G (t-test).

Table 15d

Scores of subscales of SSS-V and BIS/BAS Scales in club drug users with respect to MAO-A genotypes amongst 110 female controls. The SSS-V and BIS/BAS scores for club drug users with the three different genotypes (GG, TG, TT) were shown. Comparisons between all three genotype groups with mean scores of SSS-V and BIS/BAS subscales were carried by one-way ANOVA. The mean scores of SSS-V and BIS/BAS subscales were compared between subsamples defined by the presence or absence of alleles using Student's t-test.

Table 15d Scores of SSS-V and BIS/BAS subscales in female controls with respect to MAO-A genotypes.

MAO-A (Female controls)									
		SSS-V scores (mean±SD) [a]				BIS/BAS scores (mean±SD)			
Genotype	N	Boredom susceptibility	Disinhibition	Experience seeking	Thrill and adventure seeking	BIS	BAS reward responsiveness	BAS fun seeking	BAS drive
G/G	46	2.1±1.3	2.2±1.8	3.2±1.7	4.8±3.1	21.6±3.3	16.9±2.4	11.0±2.1	11.8±2.3
T/G	47	2.5±1.3	1.8±1.7	2.9±1.6	5.5±2.7	20.7±3.2	16.6±2.4	11.4±1.8	11.8±2.6
T/T	17	1.7±1.2	2.4±1.8	3.8±1.8	5.5±3.5	20.7±3.3	16.4±2.0	11.4±2.0	11.0±2.0
T/G+G/G	93	2.3±1.7	2.0±1.8	3.1±1.8	5.2±3.0	21.1±3.3	16.7±2.0	11.2±2.0	11.8±2.0
T/G+T/T	64	2.2±1.9	2.0±1.8	3.1±1.8	5.5±3.0	20.7±3.3	16.6±1.7	11.4±2.0	11.6±1.9
P [b]		0.21	0.44	0.18	0.55	0.37	0.68	0.64	0.36
P [c]		0.16	0.40	0.10	0.64	0.60	0.53	0.67	0.15
P [d]		0.63	0.55	0.82	0.27	0.16	0.43	0.35	0.58

[a] Personality test scores are given as T-scores, which are standardized using normative data to have a mean ± SD.

[b] Significant levels of comparisons between all three genotype groups (one-way ANOVA)

[c] Significant levels of comparisons between T/G+G/G and T/T (t-test).

[d] Significant levels of comparisons between T/G+T/T and G/G (t-test).

3.3.3 T921C Polymorphism in Exon 3 of the Human DOR (hDOR) Gene

The PCR product of 106bp showed that the TT genotype was represented by the presence of a 89 bp single band, while genotype CC was represented by the presence of a 106 bp single band. Both the 89 bp and 106 bp bands were found in the heterozygote TC sample (Fig. 25).

Table 16a shows the genotype distributions and allele frequencies of the hDOR T921C polymorphism in club drugs users and controls. It reveals that 193 controls (64%) carried the TT homozygous genotype, 19 (6%) carried the CC homozygous genotype and 91(30%) were carries of the TC heterozygote genotype. In club drugs users, it was found that there were 150 (54%) and 19 subjects (6%) with TT and CC homozygous genotype respectively whilst 96 (35%) were TC heterozygotes. Yates χ^2 analysis showed that $\chi^2_{(2)}=7.253$; $p=0.027$. Allelic frequency distribution shows that the T allele was the most common allele in both controls (79%) and club drug users (71%). The prevalence of the T allele in the control group was significantly higher ($\chi^2_{(1)}=8.131$; $p=0.004$) than that of the club drug users. It can be noted that the higher frequency of the T allele in the control group contributed to the major genotype, homozygous TT (64%) and heterozygous TC (30%) with the number of homozygote TT individuals being two-fold that of TC heterozygous (64% vs 30%). In club drug users, there is a relatively higher percentage of heterozygous TC and homozygous CC that contributed to the higher C allele frequency found (35% in club drug users vs 30% in controls) and (11% in club drug users vs 6% in controls) respectively.

Table 16b represents the scores of the SSS-V and BIS/BAS subscales for the club

drug users with the three genotypes (TT, TC, CC). No significant association between T921C hDOR genotype and the 8 subscales of personality traits was found when compared to all three genotype groups (one-way ANOVA). When genotypes were grouped together in TC+TT vs CC, to compare between subjects with a T allele versus those without a T allele, no significant associations between the T921C hDOR polymorphism and SSS-V as well as BIS/BAS scales were found. When genotypes were grouped together in TC+CC vs TT, to compare between subjects with a C allele versus those without a C allele, again no significant was found in between personality traits and groups.

Table 16c represents the scores of the SSS-V and BIS/BAS subscales for the control group with the three genotypes (TT, TC, CC). Significant association between T921C polymorphism in hDOR gene and 'BAS fun seeking' ($p=0.02$) was found with an interaction between TT and TC ($p=0.014$, $|0.11>CI>1.31|$) when all three groups were compared (one-way ANOVA). When genotypes were grouped together in TC+TT vs CC, to compare between subjects with a T allele versus those without a T allele, no significant associations between the T921C hDOR polymorphism and SSS-V as well as BIS/BAS scales were found. When genotypes were grouped together in TC+CC vs TT, to compare between subjects with a C allele versus those without a C allele, individuals with two copies of T allele scored significantly higher in that individuals with at least one of the C allele on the subscales 'BAS reward responsiveness' ($p=0.02$, $|0.09>CI>1.04|$) and 'BAS fun seeking' ($p=0.004$, $|0.22>CI>1.38|$).

3.3.4 G861C polymorphism in the serotonin receptor 1B (5-HT1B) gene

The 548 bp PCR product genotype GG was revealed by the presence of 2 bands (96bp and 452bp), while the fragment length of the PCR product was 96bp, 142bp and 310bp when the C allele was present. Heterozygotes GC were indicated by the presence of the 96bp, 142bp, 310bp and 452bp bands (Fig. 26).

Table 17a shows that 92 controls (31%) carried the GG homozygous genotype and 82 controls (28%) carried the CC homozygous genotype and 123 carried (41%) the GC genotype. For club drug users, 58 (21%) and 72 (27%) carried the GG homozygous and CC homozygous genotype respectively whilst 141 (52%) were GC heterozygous carriers. Club drug users showed a slightly higher prevalence of the C allele when compared with the control group (53% vs 48%). The G allele was the most common allele in controls (52%) while the C allele was the most common allele in club drug users (53%). However, there was no significant difference in allele frequencies between the control and club drug users (p=0.151). However, a statistically significant difference between club drugs users and controls was observed in the genotype frequency (Yates χ^2 analysis showed that $\chi^2_{(2)}=8.411$; p=0.015).

Table 17b presents the scores of the SSS-V and BIS/BAS subscales for the club drug users with the three genotypes (GG, GC, CC). No significant association between G861C 5-HT1B genotype and the 8 subscales of personality traits was found when comparing all three genotype groups (one-way ANOVA). No significant difference on personality traits was found when the genotypes were grouped together in GC+GG vs CC to compare with those with a G allele and those without a G allele or when the

genotypes were grouped in GC+CC vs GG to compare with those with a C allele and those without a C allele.

Table 17c presents the scores of the SSS-V and BIS/BAS subscales for the control group with the three genotypes (GG, GC, CC). No significant association between G861C 5-HT1B genotype and the 8 subscales of personality traits was found when comparing all three genotype groups (one-way ANOVA). No significant difference on personality traits was found when the genotypes were grouped together in GC+GG vs CC to compare with those with a G allele and those without a G allele or when the genotypes were grouped in GC+CC vs GG to compare with those with a C allele and those without a C allele.

Fig 25. The PCR product of 106bp size was subjected to *BstE*II digestion and the resulting fragments were resolved on a 4% agarose gel that was stained with ethidium bromid. A DNA marker (GeneRuler™ 50bp DNA Ladder) was loaded on the left. Homozygous TT is shown as a 89bp band, homozygous CC is shown as a 106 bp band, heterozygous is shown as 89bp and 106bp bands .

Table 16a. Genotype distributions and allele frequencies of C and T of the T921C polymorphism in exon 3 of the human DOR (hDOR) gene in club drugs users and controls. The absolute number of cases and the percentage of total (in brackets) are shown. Yates χ^2 analysis showed that χ^2=7.253; df =2; p=0.027 in the distribution of genotype frequency and χ^2 = 8.131; df = 1; p=0.004 in the allelic frequencies between club drugs users and controls

Fig. 25

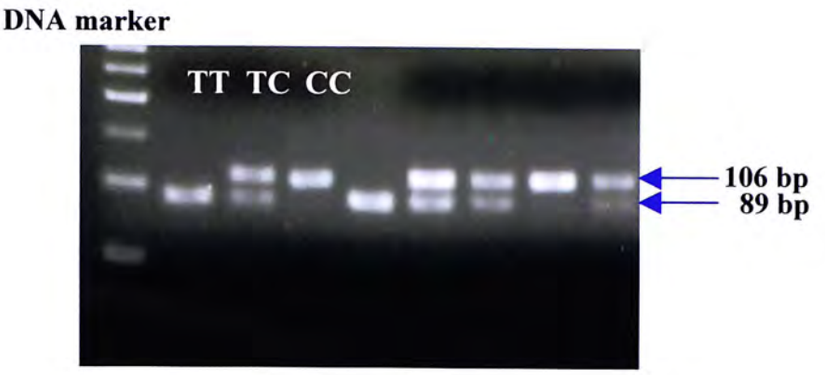


Table 16a.Genotype distributions and allele frequencies of T921C

	Genotypes, n (frequencies)				Allele frequencies		
	C/C	T/C	T/T	(p)[1]	C	T	(p)
Polydrug users (277)	31(0.11)	96(0.35)	150(0.54)		158(0.29)	396(0.71)	
Normal control (303)	19(0.06)	91(0.30)	193(0.64)	0.027	129(0.21)	477(0.79)	0.004

[1]*P*-value of Yates χ^2 analysis.

Table 16b

Scores of subscales of SSS-V and BIS/BAS Scales in club drug users with respect to hDOR genotypes amongst 277 club drug users. The SSS-V and BIS/BAS scores for club drug users with the three different genotypes (CC, TC, TT) were shown. Comparisons between all three genotype groups with mean scores of SSS-V and BIS/BAS subscales were carried by one-way ANOVA. The mean scores of SSS-V and BIS/BAS subscales were compared between subsamples defined by the presence or absence of alleles using Student's t-test.

Table 16b. Scores of the SSS-V and BIS/BAS subscales in club drug users with respect to the hDOR genotypes.

		T921C							
		SSS-V scores (mean±SD) [a]				BIS/BAS scores (mean±SD)			
Genotype	N	Boredom susceptibility	Disinhibition	Experience seeking	Thrill and adventure seeking	BIS	BAS reward responsiveness	BAS fun seeking	BAS drive
T/T	150	3.4±1.9	6.5±2.0	5.2±1.8	5.8±2.6	19.6±3.4	16.5±2.2	12.8±1.9	12.6±1.9
T/C	96	3.6±2.0	6.5±2.0	5.4±1.7	6.1±2.8	18.7±3.0	16.5±2.1	12.5±2.0	13.0±2.1
C/C	31	3.4±1.9	6.1±2.1	5.6±1.6	6.1±3.1	19.4±3.9	16.6±2.0	13.0±2.5	13.3±2.1
T/C+T/T	246	3.5±1.9	6.5±2.0	5.3±1.8	5.9±2.7	19.2±3.3	16.5±2.2	12.7±1.9	12.7±2.2
T/C+C/C	127	3.6±1.9	6.4±2.1	5.4±1.7	6.1±2.8	18.9±3.2	16.5±2.0	12.7±2.1	13.0±2.1
P [b]		0.65	0.48	0.52	0.62	0.13	0.98	0.41	0.19
P [c]		0.85	0.23	0.39	0.69	0.84	0.91	0.34	0.21
P [d]		0.45	0.66	0.31	0.33	0.08	0.85	0.63	0.09

[a] Personality test scores are given as T-scores, which are standardized using normative data to have a mean ± SD.

[b] Significant levels of comparisons between all three genotype groups (one-way ANOVA)

[c] Significant levels of comparisons between G/T+G/G and T/T (t-test).

[d] Significant levels of comparisons between G/T+T/T and G/G (t-test).

Table 16c

Scores of subscales of SSS-V and BIS/BAS Scales in club drug users with respect to hDOR genotypes amongst 303 controls. The SSS-V and BIS/BAS scores for club drug users with the three different genotypes (CC, TC, TT) were shown. Comparisons between all three genotype groups with mean scores of SSS-V and BIS/BAS subscales were carried by one-way ANOVA. The mean scores of SSS-V and BIS/BAS subscales were compared between subsamples defined by the presence or absence of alleles using Student's t-test.

Table 16c. Scores of the SSS-V and BIS/BAS subscales in controls with respect to the hDOR genotypes.

T921C (control)

		SSS-V scores (mean±SD) [a]				BIS/BAS scores (mean±SD)			
Genotype	N	Boredom susceptibility	Disinhibition	Experience seeking	Thrill and adventure seeking	BIS	BAS reward responsiveness	BAS fun seeking	BAS drive
T/T	193	2.5±1.8	2.7±2.3	3.3±1.9	5.4±2.8	20.4±3.1	16.7±2.1	11.6±2.0	11.9±2.1
T/C	91	2.5±1.6	3.0±2.3	3.5±2.0	5.7±3.1	19.8±3.1	16.2±1.8	10.9±2.0	11.9±1.9
C/C	19	2.3±1.7	2.2±2.1	3.3±1.7	5.8±2.9	19.9±3.2	15.8±2.1	11.1±1.7	11.8±1.7
T/C+T/T	284	2.5±1.7	2.8±2.2	3.4±1.9	5.5±2.9	20.2±3.1	16.5±2.0	11.4±1.9	11.9±2.0
T/C+C/C	110	2.5±1.6	2.9±2.2	3.5±1.9	5.8±3.0	19.8±3.1	16.1±2.1	10.9±1.9	11.9±1.9
P [b]		0.82	0.32	0.76	0.52	0.34	0.054	0.02	0.96
P [c]		0.55	0.25	0.82	0.65	0.68	0.15	0.51	0.79
P [d]		0.98	0.58	0.55	0.26	0.14	0.02	0.04	0.91

[a] Personality test scores are given as T-scores, which are standardized using normative data to have a mean ± SD.

[b] Significant levels of comparisons between all three genotype groups (one-way ANOVA)

[c] Significant levels of comparisons between G/T+G/G and T/T (t-test).

[d] Significant levels of comparisons between G/T+T/T and G/G (t-test).

3.3.4 G861C polymorphism in the serotonin receptor 1B (5-HT1B) gene

The 548 bp PCR product genotype GG was revealed by the presence of 2 bands (96bp and 452bp), while the fragment length of the PCR product was 96bp, 142bp and 310bp when the C allele was present. Heterozygotes GC were indicated by the presence of the 96bp, 142bp, 310bp and 452bp bands (Fig. 26).

Table 17a shows that 92 controls (31%) carried the GG homozygous genotype and 82 controls (28%) carried the CC homozygous genotype and 123 carried (41%) the GC genotype. For club drug users, 58 (21%) and 72 (27%) carried the GG homozygous and CC homozygous genotype respectively whilst 141 (52%) were GC heterozygous carriers. Club drug users showed a slightly higher prevalence of the C allele when compared with the control group (53% vs 48%). The G allele was the most common allele in controls (52%) while the C allele was the most common allele in club drug users (53%). However, there was no significant difference in allele frequencies between the control and club drug users (p=0.151). However, a statistically significant difference between club drugs users and controls was observed in the genotype frequency (Yates χ^2 analysis showed that $\chi^2_{(2)}$=8.411; p=0.015).

Table 17b presents the scores of the SSS-V and BIS/BAS subscales for the club drug users with the three genotypes (GG, GC, CC). No significant association between G861C 5-HT1B genotype and the 8 subscales of personality traits was found when comparing all three genotype groups (one-way ANOVA). No significant difference on personality traits was found when the genotypes were grouped together in GC+GG vs CC to compare with those with a G allele and those without a G allele or when the

genotypes were grouped in GC+CC vs GG to compare with those with a C allele and those without a C allele.

Table 17c presents the scores of the SSS-V and BIS/BAS subscales for the control group with the three genotypes (GG, GC, CC). No significant association between G861C 5-HT1B genotype and the 8 subscales of personality traits was found when comparing all three genotype groups (one-way ANOVA). No significant difference on personality traits was found when the genotypes were grouped together in GC+GG vs CC to compare with those with a G allele and those without a G allele or when the genotypes were grouped in GC+CC vs GG to compare with those with a C allele and those without a C allele.

Fig. 26. The PCR product of 548 bp size was subjected to *HincII* digestion and the resulting fragments were resolved on a 5% agarose gel that was stained with ethidium bromide. A DNA marker (GeneRuler™ 50bp DNA Ladder) was loaded on the left. Homozygous GG is shown as 96bp and 452bp bands, homozygous CC is shown as 96 bp, 142bp, and 310bp bands, heterozygous is shown as 96bp, 142bp, 310bp, and 452bp bands.

Table 17a. Genotype distributions and allele frequencies of G and C of the G861C polymorphism in the serotonin receptor 1B (5-HT1B) gene in club drugs users and controls. The absolute number of cases and the percentage of total (in brackets) are shown. Yates χ^2 analysis showed that $\chi^2 = 8.411$; df =2; p=0.015 in the distribution of genotype frequency and $\chi^2 = 2.064$; df = 1; p=0.151 in the allelic frequencies between club drugs users and controls.

Fig. 26

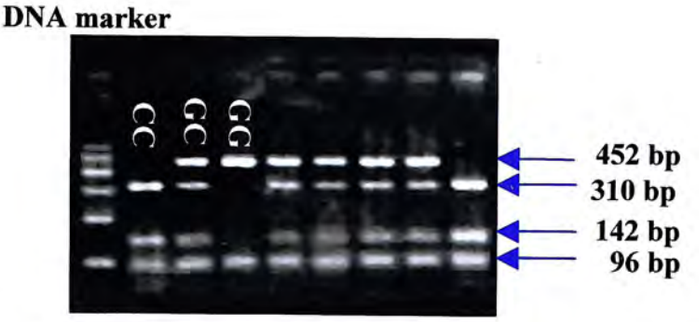


Table 17a.

Genotype distributions and allele frequencies of 5HT1B

	Genotypes, n (frequencies)				Allele frequencies		
	G/G	G/C	C/C	(p)[1]	G	C	(p)
Polydrug users (271)	58(0.21)	141(0.52)	72(0.27)		257(0.47)	285(0.53)	
Normal control (297)	92(0.31)	123(0.41)	82(0.28)	0.015	307(0.52)	287(0.48)	0.151

[1]*P*-value of Yates χ^2 analysis.

Table 17b

Scores of subscales of SSS-V and BIS/BAS Scales in club drug users with respect to 5-HT1B genotypes amongst 271 club drug users. The SSS-V and BIS/BAS scores for club drug users with the three different genotypes (GG, GC, CC) were shown. Comparisons between all three genotype groups with mean scores of SSS-V and BIS/BAS subscales were carried by one-way ANOVA. The mean scores of SSS-V and BIS/BAS subscales were compared between subsamples defined by the presence or absence of alleles using Student's t-test.

Table 17b Scores of SSS-V and BIS/BAS subscales in club drug users with respect to the 5-HT1B genotypes.

5HT1B (club drug users)									
		SSS-V scores (mean±SD) [a]				BIS/BAS scores (mean±SD)			
Genotype	N	Boredom susceptibility	Disinhibition	Experience seeking	Thrill and adventure seeking	BIS	BAS reward responsiveness	BAS fun seeking	BAS drive
G/G	58	3.3±1.7	6.6±2.0	5.3±1.7	6.4±2.6	19.6±2.3	16.6±1.9	12.6±2.3	13.0±2.1
G/C	141	3.7±2.0	6.5±2.1	5.4±1.8	6.0±2.6	18.8±3.2	16.3±2.1	12.7±2.0	12.7±2.2
C/C	72	3.3±1.8	6.5±1.9	5.2±1.7	5.5±2.9	19.7±3.9	16.8±2.3	12.8±1.6	13.0±2.2
G/C+G/G	199	3.6±1.9	6.5±2.1	5.4±1.8	6.1±2.6	19.1±3.1	16.4±2.1	12.7±2.1	12.7±2.2
G/C+C/C	213	3.6±1.9	6.5±2.1	5.3±1.7	5.8±2.7	19.1±3.4	16.5±2.2	12.8±1.9	12.8±2.2
P [b]		0.10	0.92	0.87	0.19	0.14	0.20	0.75	0.47
P [c]		0.18	0.85	0.63	0.13	0.20	0.15	0.54	0.43
P [d]		0.26	0.78	0.96	0.15	0.30	0.60	0.55	0.51

[a] Personality test scores are given as T-scores, which are standardized using normative data to have a mean ± SD.

[b] Significant levels of comparisons between all three genotype groups (one-way ANOVA)

[c] Significant levels of comparisons between G/C+G/G and C/C (t-test).

[d] Significant levels of comparisons between G/C+C/C and G/G (t-test).

Table 17c

Scores of subscales of SSS-V and BIS/BAS Scales in club drug users with respect to 5-HT1B genotypes amongst 297 controls. The SSS-V and BIS/BAS scores for club drug users with the three different genotypes (GG, GC, CC) were shown. Comparisons between all three genotype groups with mean scores of SSS-V and BIS/BAS subscales were carried by one-way ANOVA. The mean scores of SSS-V and BIS/BAS subscales were compared between subsamples defined by the presence or absence of alleles using Student's t-test.

Table 17c Scores of SSS-V and BIS/BAS subscales in controls with respect to the 5-HT1B genotypes.

5HT1B (controls)									
		SSS-V scores (mean±SD) [a]				BIS/BAS scores (mean±SD)			
Genotype	N	Boredom susceptibility	Disinhibition	Experience seeking	Thrill and adventure seeking	BIS	BAS reward responsiveness	BAS fun seeking	BAS drive
G/G	92	2.3±1.6	2.9±2.4	3.4±1.9	5.9±2.7	20.3±3.0	16.9±2.0	11.3±2.0	12.1±2.0
G/C	121	2.7±1.8	2.7±2.0	3.3±1.8	5.4±3.0	20.0±3.1	16.4±2.0	11.4±1.9	11.8±2.0
C/C	82	2.5±1.8	2.9±2.4	3.6±2.1	5.2±3.0	20.3±3.1	16.3±2.2	11.7±2.0	12.0±2.1
G/C+G/G	203	2.6±1.8	2.8±2.2	3.4±1.9	5.3±3.0	20.2±3.2	16.4±2.0	11.5±2.0	11.9±2.0
G/C+C/C	213	2.5±1.7	2.8±2.2	3.3±1.8	5.6±2.9	20.1±3.1	16.6±2.0	11.3±2.0	11.9±2.0
P [b]		0.21	0.65	0.57	0.25	0.68	0.14	0.44	0.57
P [c]		0.12	0.73	0.94	0.11	0.81	0.06	0.49	0.40
P [d]		0.95	0.52	0.32	0.29	0.51	0.22	0.20	0.81

[a] Personality test scores are given as T-scores, which are standardized using normative data to have a mean ± SD.

[b] Significant levels of comparisons between all three genotype groups (one-way ANOVA)

[c] Significant levels of comparisons between G/C+G/G and C/C (t-test).

[d] Significant levels of comparisons between G/C+C/C and G/G (t-test).

3.3.5 TaqI A Polymorphism of the DRD2 Gene

The 310 PCR product was subjected to TaqI digestion and the resulting fragments were resolved on a 2% agarose gel that was stained with ethidium bromide. Genotype A1 was revealed by the presence of a single 310bp band, whereas genotype A2 was represented by the presence of 180bp and 130bp bands. All the 130bp, 180bp and 310 bp bands were found in the heterozygote A1A2 samples (Fig. 27).

Table 18a showed that 72 controls (24%) carried the A1 homozygous genotype and 107 controls (36%) carried the A2 homozygous genotype and 107 (39%) were carriers of the A1A2 genotype. In club drug users, it was found that there were 42 (15%) and 99 (36%) having A1 homozygous and A2 homozygous genotypes respectively whilst 136 (49%) were A1A2 heterozygous carriers. A significant difference in genotype distribution was shown between controls and club drug users ($\chi^2_{(2)}$=1.344; p=0.011). A2 allele is the most prevalent allele in both controls (56%) and club drugs users (60%), however, there is no significant difference in allelic frequency (p=0.134) between the two groups.

Table 18b showed the scores of the SSS-V and BIS/BAS subscales for club drug users with the three genotypes (A1A1, A1A2, A2A2). Results showed a significant association between DRD2 genotype and BIS in BIS/BAS scale (p=0.001) with the interaction between the genotypes A1A2 and A1A1 (p=0.01, |0.33>CI>3.13|) when compared to all three groups (one-way ANOVA) with subjects homozygous for the A1 allele displaying lower BIS scores. When genotypes were grouped together in A1A2+A2A2 vs A1A1 to compare between subjects with an A2 allele versus those

without an A2 allele, individuals with two copies of A1 allele scored significantly lower than those individuals with at least one of the A2 allele on the subscale 'BIS' ($p=0.01$, $|0.45>CI>2.63|$). When genotypes were grouped together in A1A2+A1A1 vs A2A2 to compare between subjects with an A1 allele versus those without an A1 allele, no significant personality subscale scores difference was found between groups.

Table 18c showed the scores of the SSS-V and BIS/BAS subscales for controls with the three genotypes (A1A1, A1A2, A2A2). No significant association between DRD2 genotype and the 8 subscales of personality traits was found when comparing all three genotype groups (one-way ANOVA). No significant difference on personality traits was found when the genotypes were grouped together as A1A2 + A1A1vs A2A2 to compare with those with an A1 allele and with those without an A1 allele or when the genotypes were grouped as A1A2+A2A2 vs A1A1 to compare with those with an A2 allele and those without an A2 allele.

Fig 27. The 310 PCR product was subjected to *Taq*I digestion and the resulting fragments were resolved on a 2% agarose gel that was stained with ethidium bromide. A DNA marker (GeneRuler™ 50bp DNA Ladder) was loaded on the left. Homozygous A1A1 is shown as a 310bp band, homozygous A2A2 is shown as 130bp and 180bp bands, and heterozygous is shown as 130bp, 180bp and, 310bp bands.

Table 18a. Genotype distributions and allele frequencies of A1 and A2 of TaqI A polymorphism of the DRD2 gene in club drugs users and controls. The absolute number of cases and the percentage of total (in brackets) are shown. Yates χ^2 analysis showed that χ^2 =9.012; df =2; p=0.011 in the distribution of genotype frequency and χ^2 = 2.251; df = 1; p=0.134 in the allelic frequencies between club drugs users and controls.

Fig 27.

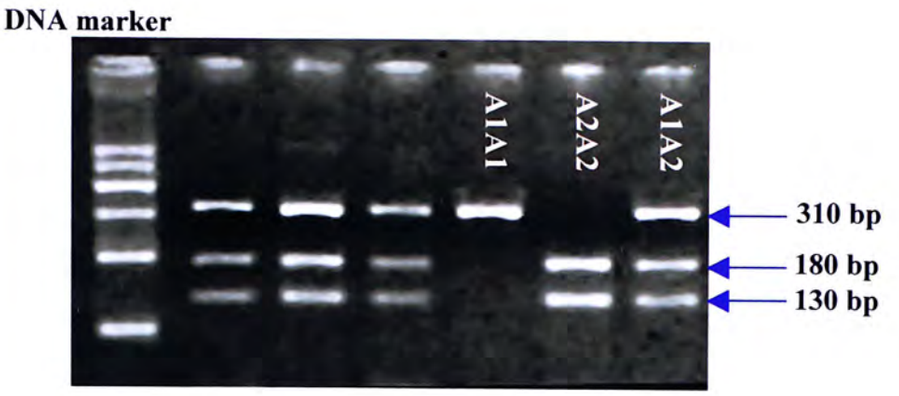


Table 18a.

Genotype distributions and allele frequencies of Taq1A

	Genotypes, n (frequencies)				Allele frequencies		
	A1A1	A1A2	A2A2	(p)[1]	A1	A2	(p)
Polydrug users (277)	42(0.15)	136(0.49)	99(0.36)		220(0.40)	334(0.60)	
Normal control (296)	72(0.24)	117(0.39)	107(0.36)	0.011	261(0.44)	331(0.56)	0.134

[1]*P*-value of Yates χ^2 analysis.

Table 18b

Scores of subscales of SSS-V and BIS/BAS Scales in club drug users with respect to DRD2 genotypes amongst 277 club drug users. The SSS-V and BIS/BAS scores for club drug users with the three different genotypes (A1A1, A1A2, A2A2) were shown. Comparisons between all three genotype groups with mean scores of SSS-V and BIS/BAS subscales were carried by one-way ANOVA. The mean scores of SSS-V and BIS/BAS subscales were compared between subsamples defined by the presence or absence of alleles using Student's t-test.

Table 18b. Scores of the SSS-V and BIS/BAS subscales in club drug users with respect to the DRD2 genotypes.

Taq1A (club drug users)									
		SSS-V scores (mean±SD) [a]				BIS/BAS scores (mean±SD)			
Genotype	N	Boredom susceptibility	Disinhibition	Experience seeking	Thrill and adventure seeking	BIS	BAS reward responsiveness	BAS fun seeking	BAS drive
A1A1	42	3.4±2.0	6.4±1.8	5.7±1.8	5.8±3.0	17.9±3.3	16.6±2.0	12.6±1.6	12.8±2.0
A1A2	136	3.5±1.7	6.5±2.0	5.3±1.6	5.8±2.8	19.7±3.4	16.3±2.2	12.8±2.0	12.7±2.1
A2A2	99	3.5±2.1	6.5±2.2	5.3±1.9	6.2±2.5	19.2±3.2	16.8±2.1	12.7±2.1	13.8±2.2
A1A2+A1A1	178	3.5±1.8	6.5±2.0	5.4±1.7	5.8±2.8	19.3±3.4	16.4±2.1	12.7±1.9	12.7±2.1
A1A2+A2A2	235	3.5±1.9	6.5±2.1	5.3±1.7	6.0±2.7	19.5±3.3	16.5±2.2	12.7±2.1	12.8±2.2
P [b]		0.94	0.90	0.38	0.42	0.01	0.29	0.84	0.43
P [c]		0.92	0.86	0.56	0.18	0.95	0.17	0.70	0.20
P [d]		0.78	0.73	0.17	0.72	0.01	0.78	0.75	0.91

[a] Personality test scores are given as T-scores, which are standardized using normative data to have a mean ± SD.

[b] Significant levels of comparisons between all three genotype groups (one-way ANOVA)

[c] Significant levels of comparisons between A1A2+A1A1 and A2/A2 (t-test).

[d] Significant levels of comparisons between A1A2+A2A2 and A1/A1 (t-test).

Table 18c

Scores of subscales of SSS-V and BIS/BAS Scales in club drug users with respect to DRD2 genotypes amongst 296 controls. The SSS-V and BIS/BAS scores for club drug users with the three different genotypes (A1A1, A1A2, A2A2) were shown. Comparisons between all three genotype groups with mean scores of SSS-V and BIS/BAS subscales were carried by one-way ANOVA. The mean scores of SSS-V and BIS/BAS subscales were compared between subsamples defined by the presence or absence of alleles using Student's t-test.

Table 18c Scores of the SSS-V and BIS/BAS subscales in controls with respect to the DRD2 genotypes.

Taq1A (controls)

		SSS-V scores (mean±SD) [a]				BIS/BAS scores (mean±SD)			
Genotype	N	Boredom susceptibility	Disinhibition	Experience seeking	Thrill and adventure seeking	BIS	BAS reward responsiveness	BAS fun seeking	BAS drive
A1A1	72	2.4±1.6	2.7±2.3	3.4±1.8	5.4±2.8	20.5±2.8	16.1±2.2	11.2±1.8	11.7±2.0
A1A2	117	2.6±1.8	2.9±2.3	3.4±1.8	5.8±3.0	20.2±3.2	16.7±2.0	11.4±1.9	12.0±2.0
A2A2	107	2.3±1.7	2.7±2.2	3.3±2.1	5.3±2.9	20.1±3.2	16.6±1.8	11.6±2.2	12.0±2.0
A1A2+A1A1	189	2.6±1.7	2.8±2.3	3.4±1.8	5.6±2.9	20.3±3.1	16.5±2.1	11.3±1.9	11.9±2.0
A1A2+A2A2	224	2.5±1.8	2.8±2.2	3.3±1.9	5.6±2.9	20.1±3.2	16.7±1.9	11.5±2.0	12.0±2.0
P [b]		0.43	0.82	0.79	0.47	0.65	0.14	0.46	0.63
P [c]		0.30	0.61	0.50	0.40	0.63	0.50	0.30	0.78
P [d]		0.79	0.90	0.85	0.66	0.35	0.05	0.28	0.34

[a] Personality test scores are given as T-scores, which are standardized using normative data to have a mean ± SD.

[b] Significant levels of comparisons between all three genotype groups (one-way ANOVA)

[c] Significant levels of comparisons between A1A2+A1A1 and A2/A2 (t-test).

[d] Significant levels of comparisons between A1A2+A2A2 and A1/A1 (t-test).

3.3.6 The A118G polymorphism in exon 1 of the human MOR (hMOR) gene

The 151 PCR product was subjected to NruI digestion and the resulting fragments were resolved on a 4% agarose gel that was stained with ethidium bromide. Genotype AA was revealed by the presence of a single 151bp band, whereas genotype GG was represented by the presence of a 129 bp band and the absence of 151 bp band. Both the 151 bp and 129 bp bands were founds in the heterozygote AG sample. The 22bp fragment was difficult to visualize because of its small size and co-migration with the similarly sized primer residue; however, detection of this fragment was not critical for genotype determination (Fig. 28).

Table 19 shows that 141 controls (47%) carried the AA homozygous genotype and 39 controls (13%) carried the GG homozygous genotype whilst 118 (40%) carried the AG genotype. In the club drug users, 117 (44%) and 34 (13%) were AA homozygous and GG homozygous respectively whilst 118 (40%) were AG heterozygous carriers. The A allele was the most common allele in both controls (67%) and club drugs users (66%). No significant difference was observed in the genotype frequency ($p=0.64$) between the controls and club drug users. When allelic frequency was examined, no significant difference between the control and club drugs users ($p=0.577$) was also found. Since no significant difference was found in the genotype and allelic frequency for the hMOR polymorphism, further correlation studies with personality trait subscales were not made.

Fig 28. The 151 PCR product was subjected to *Nru*I digestion and the resulting fragments were resolved on a 4% agarose gel that was stained with ethidium bromide. A DNA marker (GeneRuler™ 50bp DNA Ladder) was loaded on the left. Homozygous AA is shown as a 151bp band, homozygous GG is shown as 129bp, and 310bp bands, heterozygous is shown as 129bp and 151bp bands.

Table 19. Genotype distributions and allele frequencies of A and G of the A118G polymorphism in exon 1 of the human MOR (hMOR) gene in club drugs users and controls. The absolute number of cases and the percentage of total (in brackets) are shown. Yates χ^2 analysis showed that $\chi^2 = 0.894$; df =2; p=0.64 in the distribution of genotype frequency and $\chi^2 = 0.311$; df = 1; p=0.577 in the allelic frequencies between club drugs users and controls.

Fig 28.

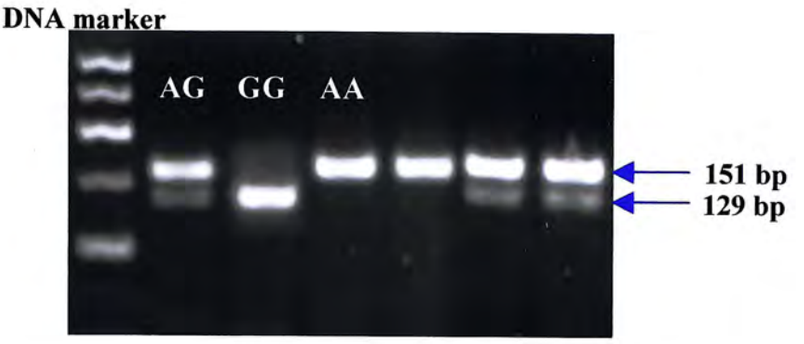


Table 19. Genotype distributions and allele frequencies of A118G

	Genotypes, n (frequencies)				Allele frequencies		
	A/A	A/G	G/G	(p)[1]	A	G	(p)
Polydrug users (267)	117(0.44)	116(0.43)	34(0.13)		350(0.66)	184(0.34)	
Normal control (298)	141(0.47)	118(0.40)	39(0.13)	0.64	400(0.67)	196(0.33)	0.577

[1]*P*-value of Yates χ^2 analysis.

3.3.7 The 44 bp insertion/deletion polymorphism in the promoter region of the serotonin transporter gene (SLC6A4)

The 44bp insertion/deletion polymorphism in the promoter region of the serotonin transporter gene (SLC6A4) was shown in Figure 29 with the short (S) allele being represented by the presence of a 484 bp single band, and the long (L) allele being represented by the presence of a 528 bp single band. Both the 484 bp and 528 bp bands were found in the heterozygote SL genotype.

Table 20 shows that 155 controls (52%) carried the SS homozygous genotype and 31 controls (10%) carried the LL homozygous genotype whilst 114 (38%) carried the SL genotype. In the club drug users, 127 (47%) and 32 (11%) carried the SS homozygous and LL homozygous genotype respectively whilst 112 (41%) were SL heterozygous carriers. The S allele was the most common allele in both controls (71%) and club drugs users (67%). No statistically significant differences were observed between club drug users and controls for genotype frequency ($p=0.511$) and allele frequency ($p=0.251$).

Since no significant difference was found in the genotype and allelic frequency for the SLC6A4 polymorphism, further correlation studies with personality trait subscales were not made.

Fig. 29. Observed alleles range from 484 bp short allele (S) and 528 bp long allele (L) of the 44bp insertion/deletion polymorphism in the promoter region of the serotonin transporter gene (SLC6A4) in club drugs users and controls were shown. A DNA marker (GeneRuler™ 50bp DNA Ladder) was loaded on the left. Homozygous LL is shown as a 528bp band, homozygous SS is shown as a 484bp band, heterozygous SL is shown as 484bp and 528bp bands.

Table 20. Genotype distributions and allele frequencies of short allele (S) and long allele (L) of the 44 bp insertion/deletion polymorphism in the promoter region of the serotonin transporter gene (SLC6A4) in club drugs users and controls. The absolute number of cases and the percentage of total (in brackets) are shown. Yates χ^2 analysis showed that χ^2 =1.344; df =2; p=0.511 in the distribution of genotype frequency and χ^2 = 1.316; df = 1; p=0.251 in the allelic frequencies between club drugs users and controls.

Fig. 29

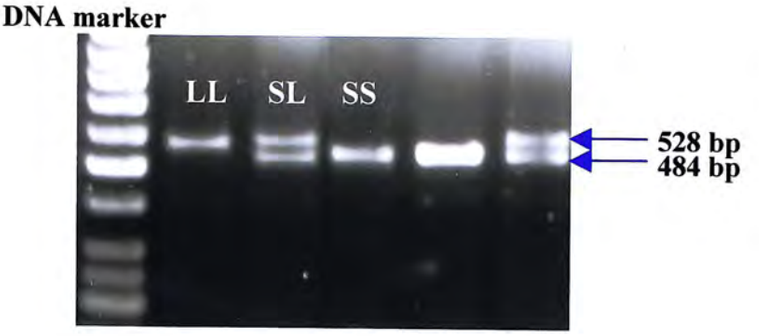


Table 20. Genotype distributions and allele frequencies of 5HTTLPR

	Genotypes, n (frequencies)				Allele frequencies		
	S/S	S/L	L/L	(p)[1]	S	L	(p)
Polydrug users (271)	127(0.47)	112(0.41)	32(0.11)		366(0.67)	176(0.33)	
Normal control (300)	155(0.52)	114(0.38)	31(0.10)	0.511	424(0.71)	176(0.29)	0.251

[1]*P*-value of Yates χ^2 analysis.

3.3.8 48 bp repeat polymorphism (DRD4) in exon 3 of the dopamine D4 receptor gene (DRD4)

The repeat allele of a 48 bp repeat polymorphism (DRD4) in exon 3 of the DRD4 gene was shown in Figure 30 with mainly the 2-repeat (2) allele being represented by the presence of a 475 bp single band, and the 4-repeat (4) allele being represented by the presence of a 619 bp single band. Both the 475 bp and 619 bp bands were found in the heterozygote 2/4 genotype.

Table 21 shows that genotype 2/2, 2/4, and 4/4 of the 48 bp repeat polymorphism in DRD4 gene were the most common repeats found in the Hong Kong Chinese populations with the relatively less proportion of genotypes 2/3, 3/4, 4/5, and 4/6 repeats presented. When we just focus the common repeat genotypes (2/2, 2/4, and 4/4), 8 controls (4%) carried the 2/2 homozygous genotype and 114 controls (61%) carried the 4/4 homozygous genotype whilst 55 (29%) carried the 2/4 genotype. In the club drug users, 10 (4%) and 157 (66%) carried the 2/2 homozygous and 4/4 homozygous genotype respectively whilst 55 (32%) were 4/7 heterozygous carriers. The 4 allele was the most common allele in both controls (78%) and club drugs users (80%). No statistically significant differences were observed between club drug users and controls for allelic frequency of short repeat (2-4 repeat) as opposed to long repeat (5-6 repeat) was $\chi^2 = 1.63$; df = 1; P-value of Yates χ^2 analysis = 0.66.

Since no significant difference was found in the genotype and allelic frequency by comparing the for the DRD4-48bp repeat polymorphism, further correlation studies with personality trait subscales were not made.

Fig. 30. Observed alleles range from 475 bp 2-repeat allele (2), 523bp 3-repeat allele (3), and 571bp 4-repeat allele (4), 619bp 5-repeat allele (5), and 667bp 6-repeat allele (6) of a 48 bp repeat polymorphism in exon 3 of the dopamine D4 receptor gene (DRD4) in club drugs users and controls were shown. A DNA marker (GeneRuler™ 50bp DNA Ladder) was loaded on the left. Genotype distributions were found mainly the homozygous 4/4, heterozygous 2/4 and homozygous 2/2 of which homozygous 4/4 is shown as a 517bp band, homozygous 2/2 is shown as a 475bp band, heterozygous 2/4 is shown as 475bp and 517bp bands. Allelic frequency of short repeat (2-4 repeat) as oppose to long repeat (5-6 repeat) was $\chi^2 = 1.63$; df = 1; *P*-value of Yatesχ^2 analysis = 0.66.

Table 21. Genotype distributions and allele frequencies of 4-repeat (4) and 7-repeat (7) of 7-repeat allele of a 48 bp repeat polymorphism (DRD4-7) in exon 3 of the dopamine D4 receptor gene (DRD4) in club drugs users and controls. The absolute number of cases and the percentage of total (in brackets) are shown. Yates χ^2 analysis showed that $\chi^2 = 1.56$; df =2; p=0.975 in the distribution of genotype frequency and $\chi^2 = 1.53$; df = 1; p=0.98 in the allelic frequencies between club drugs users and controls.

Fig. 30

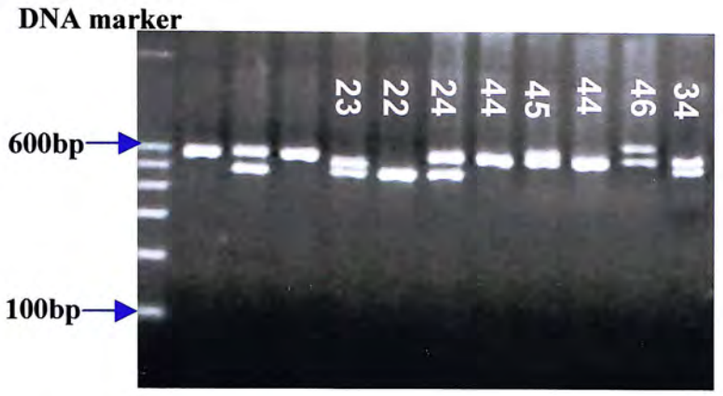


Table 21. Genotype distributions and allele frequencies of DRD4 gene

Genotype	Club drug users (n=239)	Control (n=187)
2-2	10 (0.04)	8(0.04)
2-3	1(0.004)	0
2-4	55(0.23)	55(0.29)
3-4	3(0.01)	2(0.01)
4-4	157(0.66)	114(0.61)
4-5	7(0.03)	4(0.02)
4-6	6(0.03)	4(0.02)
Allele		
2	76(0.16)	71(0.19)
3	4(0.01)	2(0.01)
4	385(0.80)	293(0.78)
5	7(0.02)	4(0.01)
6	6(0.01)	4(0.01)

3.3.9 –521C/T polymorphism in the promoter region of the dopamine D4 receptor gene (DRD4)

The 380 bp PCR product showed that the homozygous CC was represented by the presence of a 380 bp single band, whereas genotype TT was represented by the presence of the two bands, 152bp and 228bp. All the 152bp, 228bp and 380bp bands were found in the heterozygote CT sample (Fig. 31)

Table 22 shows the genotype distributions and allele frequencies of C and T of –521C/T polymorphism in the promoter region of the DRD4 gene. It reveals that 32 controls (14%) carried the CC homozygous genotype and 156 (67%) carried the TT homozygous genotype and 43 (19%) were carriers of the CT genotype. Similarly, it was found that there were 33 (12%) and 182(65%) club drugs users with CC and TT homozygous genotype respectively whilst 65(23%) were CT heterozygous carriers. Yates χ^2 analysis showed that $\chi^2_{(2)}=1.81; p=0.404$ (NS). Allelic frequency distribution shows that the T allele was the most common allele in both controls (77%) and subjects (77%). However, there was no significant different in allele frequencies between the control and subject groups (p=0.941) in the club drug users.

Since no significant difference was found in the genotype and allelic frequency for the –521C/T polymorphism in the promoter region of the DRD4 gene, further correlation studies with personality trait subscales were not made.

Fig 31 The 380 bp PCR product was subjected to *NsbI* digestion and the resulting fragments were resolved on a 4% agarose gel that was stained with ethidium bromide. A DNA marker (GeneRuler™ 50bp DNA Ladder) was loaded on the left. Homozygous CC is shown as a 380bp band, homozygous TT is shown as 152bp and 228bp bands and heterozygous CT is shown as all 152bp, 228bp and 380bp bands.

Table 22. Genotype distributions and allele frequencies of the allele frequencies of –521C/T polymorphism in DRD4 gene in the club drugs users and controls. The absolute number of cases and the percentage of total (in brackets) are shown. Yates χ^2 analysis showed that χ^2=1.81; df=2; p=0.404 in the distribution of genotype frequency and χ^2= 0.008; df = 1; p=0.941 in the allelic frequencies between club drugs users and controls.

Fig 31.

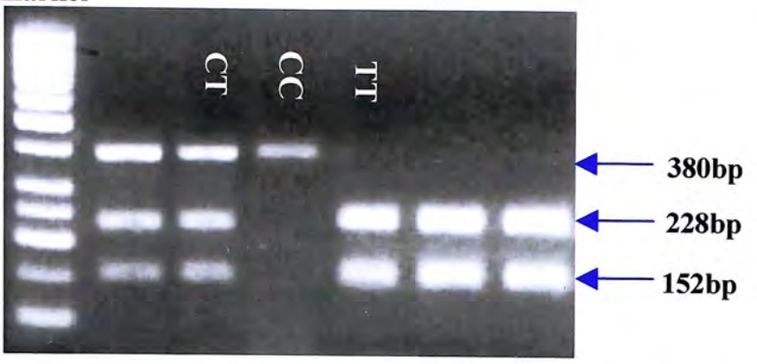


Table 22. Genotype distributions and allele frequencies of –521C/T polymorphism in DRD4 gene

	Genotypes, n (frequencies)				Allele frequencies		
	CC	CT	TT	(p)[1]	C	T	(p)
Polydrug users (280)	33(0.12)	65(0.23)	182(0.65)		131(0.23)	429(0.77)	
Normal control (231)	32(0.14)	43(0.19)	156(0.67)	0.404	107(0.23)	355(0.77)	0.941

[1]*P*-value of Yates χ^2 analysis.

Table 23.
Hardy-Weinberg Equilibrium (HWE) ratio of variant genes from both controls and club drug users were shown, including MAO-A T941G (female), COMT - val^{158}met, DRD2 - TaqI A1, DRD4 – VNTR, -C521T, 5-HTTLPR, 5-HT1B - G861C, mu-opioid receptors - A118G, and delta-opioid receptors - T921C.

Table 23. Hardy-Weinberg Equilibrium (HWE) ratio

Polymorphism	Control p-value	Club drug users p-value	Locus
MAO-A T941G (female)	0.45	0.29	Xp11.23
COMT - val^{158}met	0.01	0.37	22q11.2
DRD2 - Taql A1	0.01	0.23	11q23
DRD4 – VNTR	-	-	11p15.5
DRD4 – -C521T	0.01	0.01	11p15.5
5-HTTLPR	0.25	0.45	17q11.1-2
5-HT1B - G861C	0.02	0.15	6q13
Mu-opioid receptors - A118G	0.15	0.50	6q24-25
Delta-opioid receptors - T921C	0.21	0.07	1p36.1-34.3

CHAPTER FOUR DISCUSSION

This community-based study provides the drug use profiles of the young "club drug" users in Hong Kong as well as to investigate the association between candidate gene variants and personality traits amongst these subjects. In summary, ketamine (88.5%) followed by marijuana (83.6%), 'ecstasy' (79.8%) and 'ice' (30.1%) were the most commonly lifetime use drugs in Hong Kong (Table 2). Ketamine and 'ecstasy' were usually used in combination, with the occasional use of marijuana together with other stimulant and hallucinogenic drugs. Near to 90% and 60% of the "club drug" users have ever tried drugs during rave parties or discos in Hong Kong and in Shenzhen respectively (across the border).

4.1 Demographics and pattern of club drug use

The present study showed that there is a distinctive pattern of drug use including the local cross-border drug users in Hong Kong. Most of the "club drug" users are males with a mean age of 18.12 ± 2.475 (ranged from 13 to 29). The occupational demographics of our "club drug" users, in general, are unemployed or in lower secondary education and thus experience social and economic marginalization associated with drug use. Majority of the "club drug" users lived with both parents in public housing; their fathers (60%) are in full-time job while most mothers are housewives or in full-time job. The characteristics of the "club drug" users in Hong Kong, not surprisingly, are similar to those studies of 'ecstasy' users (Topp et al, 1999; von Sydow et al, 2002; Degenhardt, 2004) in which all of the drug users were living with their parents with lost cost housing. In the present study, the fathers of the

subjects are frequently found to be involved in several addictive behaviours including smoking (54.9%), drinking (36.1%) and gambling (33.6%) (Fig. 10).

The initial age of "club drug" use was 14.8±2.2 years (ranged from 10 to 20 years) in our study, this result did not agree with the 'floor effect' mentioned in Riley et al. (2001) (Riley et al, 2001) that represented a population who are familiar with drugs by the time they reach the legal clubbing age of 18. According to Riley et al. (2001), 'floor effect' means the people who reach the legal clubbing age of 18 are more susceptible to taking drugs since they can access drugs during clubbing. In our study, the first time drug use was found to be closely related to the education background of the "club drug" users: the educational level of "club drug" users were mainly Form 3 of lower secondary level in which most of the students finished this level at around 14-15 years old. In Hong Kong, government policy dictates that students have to receive 9 years of compulsory education (up to Form 3 level). From the present study, it seems that those youngsters who are unable to continue their study may be more susceptible to club drug use. According to the school survey study in Hong Kong, 'The 2000 survey of Drug Use among Students' (Lau, 2000), students from Hong Kong Institute of Vocational Education (IVE) schools which provide job-related training and education for the students who dropped out of school after Form 3 to pursue vocational training, were amongst the ones with the highest drug use rate. Again it showed that young people who dropped out of school after Form 3 were susceptible to drug use.

Although the 'floor effect' was not shown in our study, rave parties/ discos is the venue where the "club drug" users are most likely to take drugs with friends. Some

studies have also identified the prevalence of stimulant drugs at dance events (Forsyth, 1996; Bean et al., 1997). The present study showed that 91.7% out of the 311 (85%) club attendees have ever taken drugs during rave parties or discos in Hong Kong and 100% tried drugs when attending rave parties or discos in Shenzhen, China. Going to discos and taking club drugs in Shenzhen became a new trend amongst young people in Hong Kong over the recent years. The reasons of taking drugs in Shenzhen were similar to those reasons given for Hong Kong which are: 'to have uninhibited fun', 'to relax', and 'to seek new exciting experiences'. It was shown that "club drug" users could buy cheaper club drugs at the discos in Shenzhen when compared to Hong Kong (42% as opposed to 15% in Hong Kong, Fig 19). A significantly higher prevalence of 'ecstasy' (OR=1.91, p=0.037) and benzodiazepines use (OR=2.28, p=0.018) were found in cross-border drugs users in Hong Kong in the last 12 months when compared to those non cross-border drugs users in Hong Kong. A significantly higher prevalence of marijuana (OR=1.95, p=0.015); (OR=2.21, p=0.0016) and 'ice' use (OR=4.39, p<0.0001); (OR=2.21, p=0.009) were found in cross-border drugs users in Hong Kong in the last 12 months and lifetime use respectively when compared to the same group of cross-border drugs users taking drugs in Shenzhen. The reason for this could be marijuana and 'ice' is not the drugs usually taken during clubbing in Shenzhen, in other words, those drugs were not the common drug used among the cross-border drug users when they were taking drugs in Shenzhen. 'ecstasy' use was shown consistently to be prevalent among cross-border drug users in Hong Kong and Shenzhen during the period of lifetime, the past 12 months, and current use. It was suggested that 'ecstasy' was the common drug use among the cross-border drug users in Shenzhen, the reason for this could be related to the lower price of club drugs sold in Shenzhen. Such a trend is of concern since this will

encourage more club drug use across the border. However, the purity of the drugs, like 'ecstasy', esp. drugs from across the border, various 'ecstasy' tablets ranged from having 10%-30% MDMA as the active ingredient (Cheng et al., 2003), whereas 'esctasy' from across the border, the purity of MDMA ranged from 0.10 to 0.15 grams of MDMA.HCl per tablet.

Marijuana was the second most common "club drug" used among the "club drug" users, this is however different to that reported by the government's 51st CRDA report in which marijuana was the third common club drugs among those drug users. The reasons for this discrepancy may due to the different sources of the "club drug" users between the present study and the 51st CRDA report. In the CRDA report, 61.6% of newly reported cases were reported by law enforcement agencies, 23.9% by treatment and rehabilitation agencies, 19.7% by welfare agencies and 2.5% by hospitals and clinics while all club drugs users in our study were recruited from the community, in other words, the hidden population of "club drug" users were investigated in the present study. Our study also showed that marijuana followed by 'ecstasy', 'ice', cough mixture, benzodiazepines, and solvents were the next most common club drugs used. The present study showed a significant higher cumulative prevalence for marijuana use in males (OR=1.97, p=0.017). A similar result was also shown in a German study (Perkonigg A. et al, 1999). Warner et al., (1995) suggested that the significant gender difference observed was due mainly to the significantly higher risk for first use of marijuana among males. Moreover, it was shown in our study that the younger age group tends to be the most frequent users of marijuana suggesting that younger males may prefer using marijuana.

The most common drugs used amongst female "club drug" users were ketamine, 'ecstasy' and 'ice'. A significantly higher frequency of 'ice' use among females was reported in our present study (OR=2.28, p=0.017, Table 2), this is in agreement with that reported by Riley et al. (2001) in which Scottish females was also reported to use 'ice' significantly more frequently than males (Riley et al, 2001). In Hong Kong, the higher prevalence of "club drug" use like ketamine and 'ecstasy' and 'ice' among females could be related to the higher incidence of free "club drugs" given by friends (41.8% in females as opposed to 24.1% in males, Fig 16).

The present study showed a difference between polydrug use (more than one club drug being used at different occasions) and multiple drug use (more than one club drug used in combination at any one occasion) in our samples (92.9% having used more than one drug within the year and 77.9% of respondents having used more than one drug at any one time). Of all the participants recorded in this study, 77.8% who mixed drugs reported using between two and three drugs in their 'usual mix' with the combination of ketamine and 'ecstasy' being used together (67.2%) being the most common. 50% of the polydrug users use a combination of ketamine, 'ecstasy' and marijuana together at any one time. This polydrug use pattern found in our study was not unique, some studies have also shown that 'ecstasy' users were more likely to use marijuana, as well as being engaged in drinking and smoking (Strote et al., 2002). It was also shown that 'ecstasy' was used by adolescents who used other legal and illegal substances in a polydrug-use pattern with stronger associations towards subcultural music preferences and house-party-going (Pedersen and Skrondal, 1999).

An 18-item scale designed to measure perceived functions for substance use from

Boys et al. (2001) (Boys et al., 2001) was translated into Chinese for this study. Four of the most commonly used club drugs namely, ketamine, 'ecstasy', marijuana, and 'ice' were used in reference to the questions on 'perceived functions'. It was found that both ketamine and 'ecstasy' users shared common functions with the top five items scoring highest being: 'to help to enjoy company', 'to help to feel elated/ euphoric', 'to relax', 'to keep going on a night out with friends', and 'help to feel better when down or depressed'. Since common perceived functions for ketamine and 'ecstasy' use were found, this may perhaps explain why ketamine and 'ecstasy' were used together at any one occasion among the Hong Kong "club drug" users. Further to this, ketamine with 'ecstasy' and marijuana was the next most common combination; the top five items with the highest scores were similar to that in perceived functions for ketamine and 'ecstasy' use with the exception of 'to help to feel better when down or depressed' was replaced by 'help to sleep'. When the perceived function of 'ice' use was examined, results were quite different from the other three "club drugs" use because the main function is 'to help to stay awake' and 'help to concentrate to work or study'. The results from our study were similar to that reported by Boys et al. (2001)(Boys et al., 2001) in which the functions of "club drug" use, apart from 'ice' use, were mainly for changing mood and social purposes.

The physical symptoms shown within 24 hrs after taking "club drugs" were mainly: decrease in appetite (68.8%), poor physical coordination (53.6%) and inability to sleep (49.2%). Behavioral symptoms include: hallucination (58.3%), amnesia (49.2%), and feeling depressed or uninterested in things for more than 24 hrs among the "club drug" users who used most frequently any one of the four "club drugs" including ketamine, marijuana, 'ecstasy', and 'ice'. The above symptoms, not surprising, were

due mainly to ketamine and 'ecstasy' use. It has been shown in humans that ketamine, at subanaesthetic doses, disrupted attentional function and explicit memory (Adler et al., 1998;Krystal et al., 1998;Newcomer et al., 1999). Furthermore, some studies reported that regular, frequent use of 'ecstasy' is associated with sleep disorders, depressed mood and elevated anxiety and trait impulsiveness (Morgan et al., 1998, 2000; Parrott et al., 2000; Wareing et al., 2000). The symptoms mentioned in these studies of the frequent ketamine or 'ecstasy' users are similar to that described in our "club drugs" users, thus raising considerable alarm that these subjects are perhaps suffering from some of the long term cognitive deficits as well as psychiatric disorders reported in other studies. It seems that long term medical follow-up of these individuals is needed.

The development of potential tolerance and dependence symptoms were also shown in the present study. 72.8% "club drug" users have ever wanted to or tried to cut down on drugs but 27.7% subjects found they could not stop or cut down using drugs suggesting that these individuals may be developing dependence towards "club drug" use. 78% claimed that they needed to increase the quantity of drugs by 50% or more and 48.5% needed markedly increased amounts of drugs to get an effect or found that they could no longer get high on the amount they used to use; 40.7% have used drugs more days or in larger amounts than the "club drug" users. This suggested they were developing tolerance to these drugs. Even though dependence on ketamine was sporadically described (Hurt and Ritchie, 1994) it was suspected to be not uncommon. The present study further provides evidence that the use of ketamine may result in drug dependence. It has been shown that ketamine induce place preference using the conditioned place preference method in mice suggesting the dependence potential of

this drug (Suzuki et al., 2000). It was suggested that ketamine could indirectly activate the mesolimbic dopaminergic system by blocking the NMDA receptors which was verified to modulate the mesolimbic dopamine neurons in the VTA and NAc (Pulvirenti et al., 1991), resulting in the production of the rewarding effect since a high dose of ketamine increased dopamine turnover in several brain regions (Irifune et al., 1997). It was also shown that long-term marijuana use can lead to addiction for some people and along with craving, withdrawal symptoms like difficulty sleeping, and anxiety can make it hard for chronic marijuana users to stop using the drug (Hanson, 2002). In line with the potential that "club drugs" like ketamine and marijuana can develop tolerance and dependence, this poses considerable medical concern for the community and that the government should provide more health check-up service for these club drug youths in which such problems could be identified earlier.

When drug users were questioned on their knowledge on drugs, 20.9% and "club drugs" users did not believe that "club drugs" could cause mental problems whilst 31.3% did not believe it may be addictive or could lead to dependence. Such poor knowledge on the negative effects of drugs could result in increased frequency of drug use of "club drugs" or enhanced risky behaviour like unprotected sex. It is therefore crucial to provide continual drug education especially for the youngsters who are still at school.

The psychological status of "club drugs" users was that 91.2% of them felt stressful in their daily life of which 17% felt extremely pressurized. In our study, only 30.9% of our subjects are employed, the rest are unemployed or still at school, therefore most

of them were facing the problems of unemployment. Furthermore, 38.1% are from single homes, hence pressure come from single parent family was present among some of our "club drug" users. These factors could have an additive effect in the stresses in life that they encounter. It was shown that the initiation of smoking in adolescence is a means to cope with stressful events in life (Koval et al., 1999, 2000). Since most of our subjects are smokers, this could be related to the stressful elements in the life that they lead.

4.2 Personality traits assessment

In order to understand whether personality traits are related to the vulnerability of drug use, two personality trait scales, namely, SSS-V and BIS/BAS were used to study sensation seeking and harm avoidance behaviour respectively. Some personality traits like sensation seeking and aggressive antisocial behaviour have been shown to be predictors of drug taking, drinking or smoking (Zuckerman and Kuhlman, 2000). We therefore compared the personality traits of 360 club drug users to that of 303 controls to ascertain whether such a correlation exists for Chinese club drug users since no such information is currently available. Results showed that "club drugs" users had significantly higher sensation-seeking scores measured by both SSS-V and BIS/BAS subscales, they include: 'boredom susceptibility', 'disinhibition', 'experience seeking', 'thrill and adventure seeking' for SSS-V, and, 'BAS fun seeking', 'BAS drive' for BIS/BAS but not for 'BAS reward responsiveness' in which there was no significant difference between "club drug" users and control. This could be due to the tendency of both adolescent "club drug" users and controls focusing on the positive response to reward. For the BIS subscale of BIS/BAS scale, "club drug" user scored lower than

the controls (Table 10), the lower scores observed in the "club drug" users may be attributable to their chronic drug use. In general, our study showed that higher sensation seeking and lower anxiety scores were found in "club drug" users, these results agreed to what Wagner et al. (2001) proposed in that high sensation seeking and low anxiety are predictors of substance use (Wagner, 2001). Another study also showed that 'ecstasy' users tend to have higher sensation seeking scores (Bobes et al., 2002) thus also agreeing with the present findings. It was shown that higher anxiety or harm avoidance was associated with alcohol dependence (Lynskey et al., 1999). Our study showed a significantly lower BIS (a trait indicated anxiety) scores in "club drug" users with alcohol use suggesting these subjects may be the type II alcohol users who drink for enjoyment and for the disinhibition produced by alcohol and are more likely to be engaged in fighting and other antisocial behaviours (Cloninger, 1987b).

The mean score of 'disinhibition' in "club drug" users was more than double the score in controls; it could be explained by the presence of the 'drug-related statements' in the 10-statement 'disinhibition' subscale resulting in "club drug" users having a dramatically different score from controls.

When gender was taken into consideration (Table 11 & 12), no significant difference was found in 'thrill and adventure seeking' between female "club drug" users and female controls, in other words, the higher score observed for the 'thrill and adventure seeking' subscale in all the "club drug" users was merely contributed by the males. Furthermore, male "club drug" users were shown to have significantly higher scores in sensation seeking including, 'boredom susceptibility' ($p=0.018$), 'disinhibition'

($p<0.0001$), and lower anxiety scores, BIS ($p<0.025$) (Table 13). It may explain why there are more male than female "club drug" users observed in the general population (Gov't 51st CRDA report) as well as in our present study. Female "club drug" users in our study were shown to have significantly higher BIS scores (anxiety). A study illustrated that females showed a stronger response to 'ecstasy' when compared to males was consistent with an increased susceptibility of women to the 5-HT-releasing effects (5-HT is known to play an important role in mood and anxiety disorders) (Liechti et al., 2001) thus may explain why females respond to the chronic "club drug" uses resulting in higher anxiety scores when compared to male "club drug" users.

4.3 Gene polymorphisms

The current hypothesis is to test whether there is an association in "club drug" use with gene variants of the reward and reinforcement pathway of the brain. The hypothesis is then extended to test whether these gene variants are further associated with certain personality traits like novelty seeking, impulsivity and anxiety thus making an individual more vulnerable to "club drug" use. COMT, MAO-A, hMOR, hDOR, DRD2, DRD4, 5HT1B, and 5HTTLPR gene variants in "club drug" users and controls were examined. Polymorphisms that were investigated included the G1947A Val$^{108/158}$ Met polymorphism in the COMT gene, T941G polymorphism in the MAO-A gene, T921C polymorphism in exon 3 of the hDOR gene, A118G polymorphism in exon 1 of the hMOR gene, TaqI A polymorphism of the DRD2 gene, VNTR 48 bp repeat in exon 3 of the DRD4 gene, G861C polymorphism in the

5-HT1B gene, and the 44 bp insertion/deletion polymorphism in the 5-HTTLPR gene. Statistical significance was found between the "club drug" users and controls in the COMT val$^{108/158}$met polymorphism in both the genotype and the allelic frequency (Table 14a). For the MAO-A T941G polymorphism, a significant difference (Table 15b) in allelic frequency in the female subjects was found. For the T921C polymorphism in exon 3 of the hDOR gene, significant differences were found in both the genotype and allelic frequency (Table 16a). Genotype frequencies of the G861C polymorphism in the 5-HT1B gene (Table 17a) and the TaqI A polymorphism of the DRD2 gene (Table 18a) were also found to be significantly different between subjects and controls. However, no statistical significance was found in the polymorphisms of the hMOR, DRD4, and 5-HTTLPR genes (Tables19-22).

When the interaction between personality traits and the gene variants were examined, It was showed that G1947A val$^{108/158}$met polymorphism in the COMT gene was associated with personality traits namely 'BAS drive', 'BAS fun seeking' and 'boredom susceptibility' (Table. 14b, 14c); T941G polymorphism in the MAO-A gene was associated with personality traits, 'BIS' and 'BAS reward responsiveness' (Table. 15c, 15d); T921C polymorphism in exon 3 of the hDOR gene was associated with 'BAS reward responsiveness' and 'BAS fun seeking' (Table. 16b, 16c); and TaqI A polymorphism of the DRD2 gene was associated with 'BIS' (Table. 16b, 16c).

4.3.1 COMT G1947A, Val$^{108/158}$ Met polymorphism

COMT is an obvious candidate gene for a number of neurologic disorder that involve noradrenergic or dopaminergic systems since the gene variant G1947A is a functional

mutation that affects enzyme activity. A predominance of H alleles (G allele, high COMT activity) had been shown to be associated with substance abuse (Vandenbergh et al., 1997; Lachman et al., 1997) increase risk for schizophrenia (Egan et al., 2001) as well as ADHD impulsive-hyperactive phenotype (Eisenberg et al., 1999). On the other hand, a predominance of L alleles (low COMT activity) was shown to be associated with late-onset alcoholics (Hallikainen et al., 1999b); higher frequency of social drinking in individuals (Kauhanen et al., 2000); and higher susceptibility to obsessive-compulsive disorder or depressive disorders (Karayiorgou et al., 1997, 1999). In view of this, we have examined the Chinese "club drug" users for the COMT val/met polymorphism. The results in the present study showed that a significant association exists for the COMT val/met polymorphism in the Chinese "club drug" users. In the Hong Kong Chinese population, the H allele is more common than the L allele in both controls (73%) and "club drug" users (79%) with "club drug" users having a higher H allele frequency than the controls. The increase of H allele was due to the significant increase in HH genotype with a decrease in the number of HL genotype. The increase in H allele and in the "club drug" subjects suggested that these individuals, due to their higher COMT activity, might have an increase in dopamine catabolism resulting in lower dopamine availability in the prefrontal cortex. This in turn may generate a feedback mechanism whereby the intake of "club drugs" could help stimulate the release of dopamine in the mesolimbic pathway thus compensating for the depletion of dopamine in the prefrontal synaptic area.

There is considerable ethnic differences in the distribution of the COMT*H allele frequencies. The H allele frequency ranged from: Chinese (range, 0.72-0.78) (Xie T.

et al, 1997; Chen et al, 1997), Han Chinese (0.82) (Hinney et al., 1997), Japanese (range 0.65-0.71) (Kunugi et al., 1997; Ohara et al., 19098), Spanish (0.57) (Gutierrez et al., 1997), Caucasian (0.48) (Hoda et al., 1996), Finnish (0.42) (Syvanen et al., 1997). Our subjects and controls fall in the Chinese range, namely (0.73-0.79), reported by other studies. This suggests that the Chinese population have global higher H allele frequency and could be more predisposed to "club drug" use.

The personality trait study showed that the "club drug" users have higher sensation seeking and lower anxiety (BIS) scores when compared to the controls. When the interaction between the genotypes and that of the personality traits of the "club drug" users we studied (Table 14b), a significantly higher score in 'boredom susceptibility' (aversion to persistence of repetition) ($p=0.02$, $|0.069>CI>0.995|$) and 'BAS drive' ($p=0.01$, $|0.196>CI>1.251|$) in individuals with at least one L allele than in individuals with two copies of the H allele. When we examined the control group, there was no significant difference in 'boredom susceptibility' and 'BAS drive' scores but there was a significant difference in 'BAS fun seeking' according to the genotypes (Table 14c). Individuals with two copies of the H allele scored significantly higher than individuals with at least one of the L allele on the subscale 'fun seeking' in controls ($p=0.02$, $|-1.0>CI>-0.01|$). Since a significantly higher H allele frequency was also shown in "club drug" users, this association could reflect a relatively higher 'fun seeking' score in individuals with two copies of the H alleles. The significant difference found between "club drug" users and control could therefore be related to the chronic drug use resulting in change in personality traits in "club drug" users. A longitudinal study of these same "club drug" users will further confirm this possibility.

Benjamin et al. (2000) showed that there was a significant interaction between COMT and 5-HTTLPR in which with the presence of both COMT homozygous HH or LL genotypes and the presence of the short 5-HTTLPR allele raised 'persistence' score - a subscale of the reward dependence trait in TPQ. Those with high persistence scores were characterized as industrious, hard working, ambitious, perfectionistic in the TPQ (Benjamin et al., 2000). Therefore the lower scores of boredom susceptibility found in both homozygote HH and LL genotypes but higher scores in heterozygote HL genotype found in the present study might be under the interference of other genes. However, "club drug" users carried heterozygote HL was found to have higher sensation seeking score together with lower BIS score when compared with those with homozygote HH or LL. It may due to the heterozygote HL genotypes with the midway enzyme activity between homozygote individuals did not have the problem of excess (in LL genotype) or depletion (in HH genotype) of dopamine in prefrontal cortex.

4.3.2. MAO-A T941G polymorphism

The present study revealed that a significant difference in MAO-A genotype and allelic frequency in female "club drug" users. It seemed that the T allele might play a role in the predisposition of "club drug" use in females since there is a significant increase in allelic frequency for the T allele in the female "club drug" users. The increase in T allele was mainly due to the increase in homozygous TT genotype with a significant decrease in homozygous GG genotype in female "club drug" users. The female "club drug" users also showed a lower BIS score of the BIS/BAS personality trait assessments. When the interaction between genotype and personality traits was

assessed, it was shown that females with homozygous TT genotype were significantly associated with lower BIS score (lower anxiety) than those with homozygous GG genotype. A relatively higher BIS score was found in individuals carrying TT genotype than individual carrying homozygous GG genotype even if no significant difference was found in the control group. TT genotype is shown to be associated with lower MAO-A activity, thus individuals should have more serotonin level in brain since MAO-A was shown to have a higher potency in degrading serotonin than dopamine (Cases et al., 1995). Serotonin is the key element in the role of the behavioural inhibition mechanism mediated by the serotonergic system that originates in the medial raphe nucleus and ascending to limbic and neocortical brain structures (Zuckerman and Kuhlman, 2000) therefore disturbed serotonin metabolism in the brain has been associated with depressive mood and anxiety (Staley et al., 1998). Our female subjects have homozygous TT allele (low MAO-A activity) associated with lower anxiety scores suggesting that anxiety traits and MAO-A may be a good predictor for "club drug" use in females.

However Li et al. (2004) showed that women having higher anxiety levels may be due to the fact that estrogen inhibits COMT (Li et al., 2004). It was shown that there is an interaction between the homozygote HH COMT (high H) is also associated with lower BIS scores (Enoch et al., 2003). Therefore from our study, it was shown that there is a genetic association in anxiety traits in female "club drug" users.

4.3.3. hDOR T921C polymorphism

Mayer et al. (1997) showed that the C allele in the T921C silent polymorphism in exon 3 of the hDOR gene was significantly associated with heroin dependence

(Hinney et al., 1997). It has also been demonstrated that there is an upregulation of the DOR binding site accompanying the development of morphine tolerance and dependence in mice (Abdelhamid and Takemori, 1991). Results from our laboratory also showed an association exists with Chinese heroin-dependent subjects with heroin-dependent subjects having a higher frequency of the C allele when compared to the controls (unpublished data). Although none of our "club drug" users have ever tried opiates like heroin, the frequency of the C allele frequent was also shown to be significantly higher in the "club drug" users than in the controls. Significant difference in genotype frequency was also found in "club drug" users; there is a significantly higher number of homozygous CC and heterozygous TC individuals than controls. Since the C allele was significant different for both the heroin and "club drug" users in the Chinese population, the hDOR polymorphism may be associated with substance use in general. It may be speculated that individuals with at least one copy of C allele may be more predisposed to substance abuse. On the other hand, individuals with homozygous TT may be protected.

When the interaction of personality traits and hDOR polymorphism was studied no association was found in "club drug" users. In controls, however, individuals with two copies of T allele scored significantly higher on the subscales 'BAS fun seeking' ($p=0.004$, $|0.22>CI>1.38|$) than individuals with at least one of the C allele. Similar to that shown in COMT, the T921C polymorphism also showed a significantly higher T allele frequency in "club drug" users; this could be related to the relatively higher 'BAS fun seeking' in individual with two copies of the T alleles. Due to chronic drug use, the significant difference in 'fun seeking' score between genotypes in the T921C polymorphism was absent in "club drug" users.

Since this silent hDOR T921C polymorphism did not show any change in the receptor protein sequence (Hinney et al., 1997), a functional non-synonymous OPRD1 substitution 80T>G [Phe27Cys] was found (Blomqvist et al., 2000) that may be associate with the T921C polymorphism. Unfortunately this 80T>G polymorphism was not found in both Chinese subjects and controls (Xu et al., 2002).

However, in the animal study delta-opioid receptor mediated morphine-induced CCK-LI release in the spinal cord (Gustafsson et al., 2001) and the chronic morphine treatment in rats is associated with marked reduction in phosphorylated CREB (the active form of this transcription factor) in cortex and/or striatum (23-26%) of mu- and delta-opioid receptor KO mice, but not in kappa-KO animals. These results suggest that the endogenous opioid tone acting on mu-/delta-receptors tonically stimulate CREB activation in the brain (Garcia-Sevilla et al., 2004) as well as the emotional responses, an interactions between mu- and delta- receptors was shown knock-out mice experiment (Filiol et al., 2000). In the future, other hDOR and hMOR functional polymorphisms should be investigated to further evaluate together for their contribution to substance abuse.

4.3.4. hMOR A118G polymorphism

It was shown that when the ligand binding ability of the hMOR A118G polymorphism was studied, A118G-transfected cells bind to β-endorphin 3 times more tightly and more potently (Bond et al., 1998.). The binding and signal transduction change in the receptor may influence normal physiology of the receptor,

thus rendering an individual's vulnerability to addiction. A118G have been shown to be associated with heroin-dependence in Chinese (Szeto et al., 2001) subjects. The allele frequency of the 118G for Chinese heroin-dependent subjects is 39.5% when compared to 29.4% and in controls. It seemed that the G allele might play a role in the predisposition of heroin dependence since there is a significant increase in allelic frequency for the G allele in the heroin-dependent subjects. These frequencies are comparatively higher when compared to the Caucasian (11.5%), Hispanic (14.2%) and African-American (1.6%) control population (Bond et al., 1998).

In the present study, there was no significant difference in genotype or allelic frequency for the A118G polymorphism in the club drug users. However, the allele G frequency of our "club drug" users was 34%, falling in between that reported in the heroin-dependent and control population reported earlier.

4.3.5 DRD2 TaqI A polymorphism

The DRD2 TaqI polymorphism characteristically presents two alleles namely A1 and A2 with A1 allele carriers having lower DRD2 density and fewer binding sites in the striatum (Pohjalainen et al., 1998). These carriers also demonstrated lower glucose metabolic rates in brain regions that are rich in dopamine receptors, namely regions that participated mostly in cognitive and motivational processes (Noble et al., 1997). Moreover it was also shown that the A1 allele of the DRD2 was associated with alcoholism (Bau et al., 2000; Blum et al., 1990; Ishiguro et al., 1998; Pato et al., 1993) and cocaine dependence (Noble et al., 1993), substance abuse in general (Comings et al., 1994; Goldman et al., 1997; Uhl et al., 1993), psychostimulant-preferring

polysubstance abusers (Persico et al., 1996), smoking (Anokhin et al., 1999), compulsive-impulsive-addictive behaviour (Comings et al., 1993), schizophrenia and smoking (Comings et al., 1996) and high sensation-seeking behaviour together with alcoholism (Ratsma et al., 2001).

In the present study, a significant difference in TaqIA genotype was found between "club drug" users and controls. Although there is no significant difference in allelic frequency of the A1 allele between the "club drug" users (40%) and controls (44%), the "club drug" users however have more heterozygous A1A2 genotype and fewer homozygous A1A1 genotypes when compared to the controls. As the A1 allele of the DRD2 gene is associated with numerous disorders, fewer than half of the "club drugs" users were found to carry the A1 allele, it cannot be with total conviction that one could state that the heterozygous composition is the cause of each disorder. It could be also explained by that TaqIA polymorphism may be in linkage disequilibrium with other functional mutations nearby.

When the interaction of personality traits and genotype was performed, homozygous AlA1 genotype was found to be significantly associated with lower BIS score (lower anxiety). Individual with two copies of the A1 allele displayed lower BIS scores when compared to individuals at least one copy of the A2 allele ($p=0.01$, $|0.33>CI>3.13|$). However, this significant association was not found in control group, the lower BIS score associated with homozygous A1A1 genotype in "club drug" users could be related to the chronic "club drug" use. Our results are in agreement with that reported by David et al. (2003) in which the A2/A2 genotype was significantly associated with higher anxiety levels (David et al., 2003).

4.3.6 DRD4 48bp VNTR polymorphism

In the present study, there is no significant difference in genotype or allelic frequency between "club drug" users and controls. It is possible that the investigated polymorphism itself does not confer susceptibility to "club drug" uses, but is in linkage disequilibrium with another closely situated real susceptibility polymorphism in Chinese population. In the present study, allele 4 repeats (80%) followed by 2 repeats (16%) with no 7 repeats polymorphism present in DRD4 gene is the most frequent in the Chinese population; Chinese heroin abuser carried mostly the allele 4 repeats (71%), 2 repeats (22%), 7 repeats (0.4%) (Li et al., 2000); Japanese population also carried mostly allele 4 repeats (76.1%), 2 repeats (13.8%) but only a small proportion of 7 repeats (2.2%) (Tomitaka et al., 1999); Jews carried allele 4 repeats (67.3%), allele 2 repeats (3.1%), and allele 7 repeats (24.5%) (Kotler et al., 2000); European American carried allele 2 repeats (4.5%), 4 repeats (69.7%), and 7 repeats (18.2%); African American carried allele 2 repeats (3.8%), 4 repeats (62.8%) and 7 repeats (21.8%). Recently DRD4*7R allele was found to be associated with higher novelty seeking, independent of ethnicity, sex, or age (Benjamin et al., 1996; Ebstein et al., 1996) but the findings could not be replicated in Finnish (Malhotra et al., 1996) and Swedish (Jönsson et al., 1997). Since DRD4*7R allele was seldom found in Chinese population, the effect of DRD4*7R allele might not contribute much in Chinese population. In other words, there might be other genes contribute the factor of novelty seeking in Chinese population.

4.3.7. DRD4 -C521T polymorphism

The present study showed that there was no significant difference in -521C/T polymorphism for both genotype and allelic frequency "club drug" users (77% T allele and 23% C allele) and control (77% T allele and 23% C allele), however, the "club drug" users were shown to have higher T allele frequency than that in Chinese heroin abusers (55%) (Li et al., 2000). According to the allele database 'ALFRED' from Kidd (2003), the global allele frequency of T allele polymorphism of –C521T in DRD4 gene is various among different ethnicity: 65.2% in African; 65.2% in Irish; 67.4% in Han Chinese; 53.2% in Japanese; 76.6% in Mexican. It was shown that the allele T frequency in Hong Kong Chinese population was relatively higher when comparing with different ethnic groups. Since an approximately 40% decrease in transcription efficiency if human retinoblastoma cells which transiently expressed –521T (Okuyama et al., 2001), resulting in relatively lesser prefrontal synaptic dopamine being available and much more susceptible to Chinese population to abstain the "club drugs" to stimulate the release of dopamine in mesolimbic pathway resulting in compensating for the depletion of dopamine in the prefrontal synaptic area.

4.3.8. 5-HT1B G861C polymorphism

In the present study, although there is no significant difference in allele frequency of the 5-HT1B G861C polymorphism between "club drug" users with 53% C allele and controls with 48% C allele, a significant difference in genotype frequency was however found. The "club drug" users have a higher frequency of heterozygous GC

genotype and a lower frequency of homozygous GG genotype. It is suggested that carriers with at least one C allele may be more predisposed to "club drug" use. A Finnish study showed that antisocial alcoholics were found to have a higher frequency of the 861C (62%) allele in the 5-HT1B polymorphism G861C than Finnish controls (Lappalainen et al., 1998). Ethnic variation in the 5-HT1B G861C polymorphism allele frequency was present; Taiwanese alcohol dependence with allele C (47%) and control with allele C (43%) (Sun et al., 2002); Japanese suicide victims with allele C (50.6%) and control (49.4%) (Nishiguchi et al., 2001). It seemed that our Chinese population was placed in the relatively high allele C frequent in the 5-HT1B G861C polymorphism when comparing to the other Asian countries. It was suggesting that Chinese might be more susceptible to be alcoholic or commit suicide. However, in the present study, when the interaction of the genotypes to personality traits was studied, there was no significant difference found in both "club drug" users and controls. It might be suggesting the present of a linkage disequilibrium with other known or unknown polymorphisms that affect gene expression or function of gene product.

4.3.9. 5-HTTLPR SLC6A4 44 bp insertion/deletion polymorphism

The 44bp insertion/deletion polymorphism in 5-HTTLPR gene has been also found associated with affective illness and anxiety related traits (Collier et al., 1996; Lesch et al., 1996; Ogilvie et al., 1996; Neumeister et al., 2002). Gelernter et al. (1997) showed that there is a significant difference in the short (S) allele frequency amongst European American (40.4%), African American (25.4%), and Japanese (80.2%) samples in the 44bp insertion/deletion polymorphism in 5-HTTLPR gene. Individuals

(Finnish sample) with higher S alleles were shown to have a reduction in transcription efficiency and it was found higher S alleles is associated with type 2 alcoholics (53%) characterized by having antisocial-aggressive traits; and with individuals who have early onset and familial history of alcoholism as compared to type 1 alcoholics (40%) (Hallikainen et al., 1999a). Moreover, the S allele was also reported to be associated with high novelty seeking and low harm avoidance of the anti-social alcoholics (Hallikainen et al., 1999) and is also higher in heroin-dependent subjects (west European Caucasians with S allele 51.5%) (Gerra et al., 2004).

In the present study, there was no significant difference between the genotype and allelic frequency between the "club drug" users and controls. At present, the "club drug" users and controls were shown to have 67% S alleles and 37% L allele in 5-HTTLPR. However, it was shown that Chinese population carries relatively higher frequency of S allele when comparing with those populations in western country. It implicate that Chinese might be more susceptible to be alcoholic, heroin abuse and antisocial (S allele-related traits).

Although no significant genotype distribution and allele frequency difference found in the present study, there might be other genes interact with 5-HTTLPR, for example, a significant interaction between 5-HTTLPR polymorphism and DRD4 VNTR was also found, with individuals bearing homozygous for the 7-repeat of the DRD4 genotype together with homozygous SS 5-HTTLPR genotype having higher mean scores for harm avoidance behaviour in TPQ questionnaire (Szekely et al., 2004). However, in the present study, no 7 repeat allele in DRD4 found in the "club drug" users, therefore some other genes might be taken part in affecting the expression of 5-HTTLPR.

In the present study, it can be concluded that the H allele in COMT G1947A, val$^{108/158}$met polymorphism and C allele of T921C polymorphism of hDOR gene are both associated with "club drug" use. Moreover, significant association was found between T allele in T941G polymorphism in MAOA-gene in female "club drug" users. A significant difference in genotype distribution of TaqI A polymorphism in DRD2 gene and G861C polymorphism in 5-HT1B gene were also found. It has been shown that T941G polymorphism in MAO-A gene and TaqI A polymorphism in DRD2 gene were associate with the personality trait 'BIS' (anxiety trait) in the "club drug" users whereas COMT G1947A, val$^{108/158}$met polymorphism and TaqI A polymorphism in DRD2 gene were associate with the personality trait 'fun seeking' in control groups. It is therefore concluded from the present study that both gene variants and personality traits were associated with "club drug" use. It might also suggest that multi-gene was contributes to a certain extent and the cumulative effects of their functional change might generate a more robust influence. Other potential candidate genes nearby the associated polymorphism genes found in the present study are worth to be investigated for the linkage disequilibrium. However, bias is always found in the genetic association studies as shown in a meta-analysis study of a subset of 25 associations with approximately 12 potential replication studies per association (Lohmueller et al., 2003). It was shown that false positive associations are abundant in the literature and the genetic effect in an association study will be biased upward with the original reported associations being false positive reports, conditional on that study being the first to reach statistical significance and be published. Even if there was ethnic and population stratification among the false positives association, small sample sizes could be the issue of a lack power to reliably detect the genetic effects.

Another study revealed that gene-based approach (genes are highly consistent across diverse human populations) in genetic association studies could avoid population differences usually found in the SNP-based, haplotype, and functional variant studies (Neale and Sham, 2004). In order to improve the present genetic association study, more explicit phenotype criteria, precise localization of the polymorphisms of interest, low genotyping error rate, larger sample size will be needed.

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Appendix 1a (Hong Kong version)

你好！我係 XXX 服務處的研究員，我們現在正與香港中文大學合作進行一項「香港青少年濫用藥物情況研究」。希望你可以幫我做一份問卷，好使我們對這個題目有深入的瞭解；而資料內容只會用作分析研究，並會絕對保密。多謝你的協助！

訪問員：_____ 訪問日期：_____ 問卷編號：_____

個人基本資料

1. 性別　　1) ☐ 男　　2) ☐ 女

2. 你幾多歲? _____

3. 你現時住在哪區? _____

4. 是甚麼類型的房屋?
   1) ☐ 臨時房屋
   2) ☐ 租住床位
   3) ☐ 公屋
   4) ☐ 租住私人樓宇
   5) ☐ 居者有其屋計劃
   6) ☐ 私人樓宇
   7) ☐ 其他(請註明):_____

5. 你的教育程度是
   1) ☐ 小學
   2) ☐ 中一
   3) ☐ 中二
   4) ☐ 中三
   5) ☐ 中四
   6) ☐ 中五
   7) ☐ 預科
   8) ☐ 其他(請註明):_____

6. 你現時的職業是
   1) ☐ 全職工作
   2) ☐ 兼職工作
   3) ☐ 待業
   4) ☐ 學生
   5) ☐ 輟學
   6) ☐ 其他(請註明):_____

7. 個人習慣:

	有	間中	開始年齡	沒有
吸煙	☐	☐	_____	☐
飲酒	☐	☐	_____	☐
賭搏	☐	☐	_____	☐
毒品	☐	☐	_____	☐

14.你母親的籍貫是:_____
☐ 不知道

15.你母親的工作狀況是：
1) ☐ 全職工作
2) ☐ 兼職工作
3) ☐ 待業
4) ☐ 家庭主婦
5) ☐ 退休
6) ☐ 其他(請註明):_____

16. 你母親的教育程度是：
1) ☐ 未接受過正規教育
2) ☐ 小學
3) ☐ 中學
4) ☐ 大學
5) ☐ 其他(請註明):_____

17.你母親的個人習慣:

	有	沒有	間中
吸煙	☐	☐	☐
飲酒	☐	☐	☐
賭搏	☐	☐	☐
毒品	☐	☐	☐

18.a 你家中成員有冇是濫用藥物?
1) ☐ 有(請註明關係):_____
2) ☐ 冇
3) ☐ 不知道

18.b 你或你家中成員曾否患精神病（如: 憂鬱症，焦慮症，精神分裂症，恐慌症等等……)?
1) ☐ 有(請註明關係和病稱):_____
2) ☐ 冇
3) ☐ 不知道

濫用藥物趨勢

19. 除了在香港，你有否在其他地方（例如深圳、中國其他地方或外國）曾經使用藥物?（可選多項）
   1) ☐ 深圳
   2) ☐ 廣東省
   3) ☐ 中國其他省份(請註明)：_____
   4) ☐ 其他國家(請註明)：_____
   5) ☐ 否（↳第 21 題）

20. 根據 19 題所選的地方中，你最常使用藥物的地方是?
   1) ☐ 深圳
   2) ☐ 廣東省
   3) ☐ 中國其他省份(請註明)：_____
   4) ☐ 其他國家(請註明)：_____

(如有回答第 20 題者，請在作答第 21 至 31 題時，答案必須分別填在以下兩欄中。如沒有在香港以外地方使用藥物，只須填寫欄一)

	欄一 **在香港**	**欄二** **在 20 題所選的地方**

21. 你有冇曾經去過的士高或 Rave Party?
   1) 過去 6 個月內曾經去過 ☐ ☐
   2) 6 個月前曾經去過 ☐ ☐
   3) 否 ☐ ☐
   (↳第 27.b 題)

22. 你第一次去的士高或 Rave Party 在多久之前?
   _____年 _____年
   _____月之前 _____月之前
   忘記了 ☐ ☐

23. 過去半年，你大約去過多少次的士高或 Rave Party?
   (如答 0 次，↳第 26 題)
   ☐☐☐ 次 ☐☐☐ 次
   1) 一日來回 ☐☐ 次
   2) 過一夜 ☐☐ 次；通常星期 ☐ 返上去
   3) 若逗留超過一晚，你(過去半年)平均去過多少次
   _____

24. 過去半年,你通常與甚麼人一齊去的士高或 Rave Party?(可選多項)

1) 同學	☐	☐
2) 朋友	☐	☐
3) 男/女朋友	☐	☐
4) 同事	☐	☐
5) 父母	☐	☐
6) 兄弟姊妹	☐	☐
7) 其他親戚	☐	☐

8) 自己 ☐ ☐

	在香港	在 20 題所選的地方
25. 過去半年,你通常每次會與多少人一齊前往的士高或 Rave Party? (如自己上填 0 人)	☐☐☐ 人	☐☐☐ 人

26.你去的士高或 Rave Party 的原因是 (可選多項)

1) 可以尋找新刺激	☐	☐
2) 可以結識女朋友/男朋友	☐	☐
3) 個人可以毫無拘束地玩樂	☐	☐
4) 可以較便宜地買到藥物	☐	☐
5) 可以徹底放鬆	☐	☐
6) 其他(請註明): _____	☐	☐

27a.你有否在的士高或 Rave Party 使用過以下藥物? 有 ☐(↳第 27c 題) 否 ☐

27b.你有否使用過以下藥物? 有 ☐(↳第 27c 題) 否 ☐(↳第 32 題)

27c

	曾經服食過 ("√" = 是/ "X" = 否/ "?" = 唔知)		過去一年曾服食過 ("√" = 是/ "X" = 否/ "?" = 唔知		次數 (過去三十天內)		最常用的服用方式 (如: 服食/注射/鼻吸等)		每次平均總服用量 (如: 粒/包/ 樽等)	
	在香港	在 20 題所選的地方	在香港	在 20 題所選的地方	在香港	在 20 題所選的地方	在香港	在 20 題所選的地方	在香港	在 20 題所選的地方
K 仔										
搖頭丸										
冰										
大麻										
酒							-	-		
煙							-	-		
安定										
藍精靈										
十字架										
咳水										
天拿水										
其他										

	在香港	在 20 題所選的地方
28. 你通常每次花多少錢買這些藥物?	$_____	$_____
免費，其他人請	☐	☐

29. 你從哪裏獲得上述藥物? (可選多項)

1) 朋友	☐	☐
2) 黑社會兄弟	☐	☐
3) 同學	☐	☐
4) 藥房門市	☐	☐
5) 網 Bar 內之拆家	☐	☐
6) 的士高或 Rave Party 之拆家	☐	☐
7) 自己由香港買上深圳	☐	☐
8) 家人	☐	☐
9) 其他(請註明)：_____	☐	☐

30. 在哪裏服用? (可選多項)

1) 自己屋企	☐	☐
2) 朋友屋企	☐	☐
3) 街角	☐	☐
4) 遊戲機中心	☐	☐
5) 卡拉 OK	☐	☐
6) 公廁	☐	☐
7) 公園	☐	☐
8) 其他地方(請註明):_____	☐	☐

31. 你有冇曾經試過因服用藥物而感不適並被送往醫院?

1) 曾經試過	☐	☐
2) 未曾試過	☐	☐

第一次濫用藥物之經驗

32. 你有否曾經使用過最少一次上述藥物 (Q 27c，除了煙，酒之外)

1) ☐ 有
2) ☐ 否 (↳第 61 題)

33. 你第一次服用以上所提及的藥物在多久之前? _____年 _____月之前

34. 你從哪裡取得這些藥物?

1) ☐ 朋友
2) ☐ 黑社會兄弟
3) ☐ 同學
4) ☐ 藥房門市
5) ☐ 網 Bar 內之柝家
6) ☐ 的士高或 Rave Party 之柝家
7) ☐ 自己由深圳帶返港
8) ☐ 家人
9) ☐ 其他(請註明)：_____

35. 由第一次開始，你是否經常服用以上所提及的藥物?

1) ☐ 經常服用
2) ☐ 間中服用
3) ☐ 很少服用
4) ☐ 自第一次後從不服用 (↳第 37 題)

36. 多久會服用一次?

1) ☐ 一日 1 次
2) ☐ 一星期 1-6 次
3) ☐ 一個月 1-3 次
4) ☐ 一個月少過 1 次
5) ☐ 不定時服用

37. 你第一次服用藥物的原因是 (可選多項)

1) ☐ 服後覺得自己變得成熟，並可向別人炫耀
2) ☐ 尋求快感及樂趣
3) ☐ 朋輩影響
4) ☐ 想追上潮流
5) ☐ 解悶及舒緩情緒低落問題
6) ☐ 對服用藥物的經驗感到好奇
7) ☐ 逃避問題
8) ☐ 舒緩神經
9) ☐ 其他原因 (請註明):_____

服用藥物的原因

38. 你最常服用哪一種藥物?_____

39. 你有否試過同時服用超過一種藥物?

1) ☐ 有 ⟶ 試過同時服用哪幾種? (並依照服用次數列出，最多次數先寫)

1.1)_____
1.2)_____
1.3)_____

2) ☐ 否

40. 你最近服用藥物係幾時?

_____年_____月

41.a) 你購買藥物的主要經濟來源是:

1) ☐ 主要是靠工作入息
2) ☐ 主要是向家人借
3) ☐ 主要是向朋友借
4) ☐ 主要是靠非法途徑
5) ☐ 主要是靠工作入息和向別人借
6) ☐ 主要是靠工作入息和從事非法途徑
7) ☐ 主要是向別人借和從事非法途徑
8) ☐ 免費，其他人請（↳第 43 題）
9) ☐ 主要是靠零用錢
10) ☐ 其他原因（請註明):_____

41.b) 每月大概有多少元零用及收入:$_____

42．你估計每月花多少錢在購買藥物?

$_____

43. 你最常服用的這種藥物(第 38 題)會否令你產生以下效果?（可選多項）

1) ☐ 當我情緒低落或沮喪時，會令我感到舒服一點
2) ☐ 使我感到不需擔心問題
3) ☐ 幫我鬆弛自己
4) ☐ 使我感到情緒高昂或興奮
5) ☐ 令我爛醉如泥或興奮得不能自製
6) ☐ 性交時加倍興奮
7) ☐ 使我毫無睡意
8) ☐ 幫我減肥
9) ☐ 幫我入睡
10) ☐ 使我能夠與朋友一起享樂
11) ☐ 使我更有信心或有能力去與人交談
12) ☐ 使我不再顧忌
13) ☐ 使我整晚與朋友外出玩樂
14) ☐ 幫我專心工作或讀書
15) ☐ 提高活動(包括音樂感或跳舞)的表現
16) ☐ 令我做事時減少沈悶的感覺
17) ☐ 改善服用其他藥物的效果
18) ☐ 停止因服用其他藥物後而所產生的效果

服用藥物後的徵狀

44. 服用過第 38 題所述的藥物後，在二十四小時內有冇發現以下癥狀? (可選多項)

44.a 身理癥狀

1) ☐ 食慾增加或減少
2) ☐ 動作不協調，搖晃不定
3) ☐ 失眠
4) ☐ 手心出汗或手震
5) ☐ 頭暈，頭痛
6) ☐ 不規則心跳
7) ☐ 噁心嘔吐
8) ☐ 過度活躍 (如跳舞等)

44.b 行爲癥狀

1) ☐ 失憶
2) ☐ 幻覺 2.1)☐ 聽幻覺 2.2)☐視幻覺 2.3)☐ 觸幻覺 2.4)☐ 嗅幻覺
3) ☐ 對事物沮喪或不感興趣超過一天
4) ☐ 對別人猜疑或妄想超過一天
5) ☐ 影響與家人或朋友的關係
6) ☐ 影響工作或上學表現
7) ☐ 神經過敏或容易緊張超過一天
8) ☐ 痙攣
9) ☐ 暴力傾向
10) ☐ 自殺傾向

服用藥物的潛在耐忍性或依賴性

	有	冇
45. 你有冇在一個月或以上的時間內每天用很多時間去購買、服用藥物或從藥物的作用中恢復過來?	☐	☐
46. 你有冇希望或嘗試停止服用藥物?	☐	☐
47. 你有冇發現自己不能停止服用藥物?	☐	☐
48a 你有冇需要增加服用藥物的份量，否則就不會出現預期效果?	☐	☐
(↳第 49 題)		
48b 有冇比平常服用份量多出一半或以上?	☐	☐
49. 你有冇因服用藥物而經常減少出席與家人或朋友的重要聚會及工作?	☐	☐
50. 你有冇經常服用多於之前擬定的藥物份量或服用日數?	☐	☐

	有	冇
51. 停止服用藥物後，有冇出現以下問題(可選多項)		
1) 沮喪	☐	☐
2) 緊張或不安	☐	☐
3) 疲勞或倦意	☐	☐
4) 失眠	☐	☐
5) 食慾增加或減少	☐	☐
6) 打顫或痙攣	☐	☐
7) 流汗或發燒	☐	☐
8) 噁心嘔吐	☐	☐
9) 肚瀉或胃痛	☐	☐
10) 流眼水或鼻水	☐	☐
11) 打呵欠	☐	☐
12) 心跳加速	☐	☐
52. 停止服用藥物後，有冇同時出現兩個或以上在第 51 題的癥狀?	☐	☐
53. 你有冇因減低或消除斷癮癥狀而服在第 38 題的藥?	☐	☐
54a 除了斷癮癥狀外，有冇因服用藥物後的其他健康問題?	☐	☐

請註明:
_____

(↳第 55a 題)

54b 當發現這些健康問題後，你有冇繼續服用藥物?	☐	☐
55a．你的家庭、朋友、上司、同事 或同學有冇曾經對你服用藥物反映不滿?	☐	☐

(↳第 56 題)

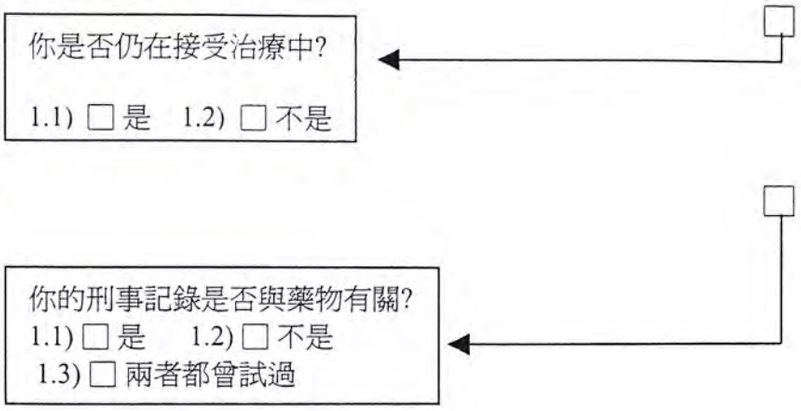
55b. 當知道因服用藥物後所帶來的問題後，你有冇繼續服用藥物?	☐	☐
56. 你有冇因服食藥物後，產生興奮感覺或其他效果而影響在上學，上班或做家務時的表現?	☐	☐
57a. 服食藥物時，你有冇發現任何心理問題，如沮喪、猜疑、胡思亂想、聽幻覺、嗅幻覺、觸幻覺、視幻覺或神經質? (請列明癥狀)	☐	☐

1)_____
2)_____
3)_____

(↳第 58 題)

57b. 當你發現這些徵狀時，你有冇停止服食藥物?	☐	☐
58. 你有冇因受服食藥物的影響而增加受傷的機會，如在駕駛時、使用刀或機械時、過馬路、攀山或游泳時?	☐	☐

	有	冇
59. 你有冇接受任何有關濫用藥物的入院治療?	☐	☐
60. 你有冇任何刑事記錄?	☐	☐



對濫用藥物之認識

61. 請講出 3 種你認識最深之藥物：
    1) _____
    2) _____
    3) _____

62. 你第一次從哪裏認識這些藥物?
    1) ☐ 電視/電台/電影
    2) ☐ 朋友
    3) ☐ 社工
    4) ☐ 報紙
    5) ☐ 父母
    6) ☐ 學校
    7) ☐ 其他(請註明)：_____

	會	不會	不知道
63. 你認爲以上所提及的藥物會否對身體造成傷害?	☐	☐	☐
64. 你認爲以上所提及的藥物會否令到精神有毛病?	☐	☐	☐
65. 你認爲以上所提及的藥物會否令人上癮及產生依賴性?	☐	☐	☐
66. 你認爲部份你所服用的藥物會否含有雜質?	☐	☐	☐
67. 你認爲以上所提及的藥物會否造成身體永久性破壞?	☐	☐	☐
68. 你認爲以上所提及的藥物會否導致死亡?	☐	☐	☐
69. 你認爲以針筒注射以上所提及的藥物會否感染愛滋病?	☐	☐	☐

心理狀況

70 你覺得平時壓力有多大?

1) ☐ 非常大壓力
2) ☐ 有相當壓力
3) ☐ 少許壓力
4) ☐ 毫無壓力

71 你覺得你能控制生活嗎?

1) ☐ 完全控制
2) ☐ 部份控制
3) ☐ 不清楚
4) ☐ 部份失控
5) ☐ 完全失控

72 你滿足現在的生活嗎

1) ☐ 非常滿足
2) ☐ 滿足
3) ☐ 不清楚
4) ☐ 不滿足
5) ☐ 非常不滿足

問卷完成，謝謝你！

Appendix 1b (English translated version for Hong Kong only)

Interviewer：_____ Date：____/____/____ Questionnaire No.:_____

Hello! I am a researcher of XXX organization. We are currently conducting a study in collaboration with the Chinese University of Hong Kong on psychostimulant use among the youths in Hong Kong. I sincerely hope that you can help us complete this questionnaire in order we can have a better understanding of the current trend of psychostimulant use. The information you provide will be kept in the strictest confidence and will only be used for the purpose of this study. Thank you for your cooperation!

Personal information

1. Sex: 1) ☐ M 2) ☐ F

2. Age:_____

3. District of residence: _____

4. Type of housing:
   1) ☐ Temporary housing/squatter
   2) ☐ Rented bed
   3) ☐ Public housing
   4) ☐ Rented private housing
   5) ☐ Home Ownership Scheme
   6) ☐ Self-owned private housing
   7) ☐ Others; please specify:_____

5. Education level:
   1) ☐ Primary school
   2) ☐ Form 1
   3) ☐ Form 2
   4) ☐ Form 3
   5) ☐ Form 4
   6) ☐ Form 5
   7) ☐ Form 6/7
   8) ☐ Others; please specify:_____

6. Work status:
   1) ☐ Full time work
   2) ☐ Part time work
   3) ☐ Unemployed
   4) ☐ Student
   5) ☐ Drop out of school
   6) ☐ Others; please specify:_____

7. Personal habits:

	Yes	Never	Occasionally
Smoking	☐	☐	☐
Drinking	☐	☐	☐
Gambling	☐	☐	☐
Substance Use	☐	☐	☐

8. At present, who do you live with?
   1) ☐ Parents
   2) ☐ Single parent (☐Father ☐ Mother)
   3) ☐ Relatives
   4) ☐ Friends
   5) ☐ On one's own
   6) ☐ Don't have a fixed address
   7) ☐ Others; please specify_____

9. Parents' marital status?
   1) ☐ Married
   2) ☐ Separated
   3) ☐ Divorced
   4) ☐ Remarried
   5) ☐ Father and/or mother deceased

10. Name the province of origin of your father's family:_____ ☐ I don't know

11. Father's work status:
    1) ☐ Full time work
    2) ☐ Part time work
    3) ☐ Unemployed
    4) ☐ Retired
    5) ☐ Others; please specify:_____

12. Father's education level:
    1) ☐ Never received any education
    2) ☐ Primary school
    3) ☐ Secondary school
    4) ☐ University
    5) ☐ Others; please specify:_____

13. Father's personal habits:

	Yes	Never	Occasionally
Smoking	☐	☐	☐
Drinking	☐	☐	☐
Gambling	☐	☐	☐
Substance Use	☐	☐	☐

14. Name the province of origin of your mother's family:_____ ☐ I don't know

15. Mother's work status :
    1) ☐ Full time work
    2) ☐ Part time work
    3) ☐ Unemployed
    4) ☐ Housewife
    5) ☐ Retired
    6) ☐ Others; please specify:_____

16. Mother's education level:
    1) ☐ Never received any education
    2) ☐ Primary school
    3) ☐ Secondary school
    4) ☐ University
    5) ☐ Others; please specify:_____

17. Mother's personal habit:

	Yes	Never	Occasionally
Smoking	☐	☐	☐
Drinking	☐	☐	☐
Gambling	☐	☐	☐
Substance Use	☐	☐	☐

18a. Does any member of your family have a history of substance abuse?
    1) ☐ Yes (Relationship):_____
    2) ☐ No
    3) ☐ I don't know

18b. Does any member of your family (including yourself) have a history of mental illness?
    1) ☐ Yes (Relationship and name of illness):_____
    2) ☐ No
    3) ☐ I don't know

Pattern of drug use

19. Have you ever taken drugs outside of Hong Kong? (For example, Shenzhen, other places in Mainland China, or some foreign countries) (You can check more than one box)
    1) ☐ Shenzhen
    2) ☐ Guangdong province
    3) ☐ Other provinces in China; please state : _____
    4) ☐ Other countries; please specify : _____
    5) ☐ Never (Go to Q21)

20. Please specify the place where you most often take drugs among the places you specified in Q19.
    1) ☐ Shenzhen
    2) ☐ Guangdong province
    3) ☐ Other provinces in China; please specify : _____
    4) ☐ Other countries; please specify : _____

(If you have answered Q20, please filled in both columns from Q21 to Q31)
(If you have taken drugs only in Hong Kong please just fill in the left column)

	Column 1 (In Hong Kong)	Column 2 (Place specified in Q20)
21. Have you ever been to any disco or rave party?		
1) Within the past 6 months	☐	☐
2) More than 6 months ago	☐	☐
3) Never (Go to Q27b)	☐	☐
22. When was the first time you went to a discos or rave parties?		
	_____(Yr) _____(Mth) ☐ Forgot	_____(Yr) _____(Mth) ☐ Forgot
23. During the last 6 months, approximately how many times did you go to the discos or rave parties? (If never, go to Q26)	☐☐☐ times	☐☐☐ times

1) Day trip ☐☐ times
2) Stay overnight ☐☐ times; which day of the week do you usually go there? _____
3) Stay more than one night _____times

	Column 1 (In Hong Kong)	Column 2 (place specified in Q20)
24. During the last 6 months, who did you go to the discos and rave parties with? (You can check more than one box)		
1) Classmates	☐	☐
2) Friends	☐	☐
3) Boy/Girlfriend	☐	☐
4) Colleagues	☐	☐
5) Parents	☐	☐
6) Brothers/Sisters	☐	☐
7) Other relatives	☐	☐
8) Alone	☐	☐

25. During the last 6 months, how many people on average each time did you go to the discos and rave parties with? (If you go alone, please fill in "0") ☐☐☐ People

	Column 1 (In Hong Kong)	Column 2 (place specified in Q20)

26. What is/are the reason(s) for going to discos or rave parties? (You can check more than one box)

1)	To seek new exciting experiences	☐	☐
2)	To find new boy/girlfriend	☐	☐
3)	To have uninhibited fun	☐	☐
4)	To buy cheaper drugs	☐	☐
5)	To relax	☐	☐
6)	Others; please specify: _____	☐	☐

27a. Have you ever taken the following drugs listed in Q27c in the discos or rave parties?

☐ Yes (Go to Q27c) ☐ No

27b. Have you ever taken the following drugs listed in Q27c?

☐ Yes (Go to Q27c) ☐ No (Go to Q32)

27c.

	Drug(s) ever tried		Drug(s) taken (Past 12 months)		Frequency. (Past 30 days)		Route of administration		Total quantity (e.g. No. of pills, packets, bottles)	
	H.K.	Q20	H.K.	Q20	H.K.	Q20	H.K.	Q20	H.K.	Q20
Ketamime										
Ecstasy										
Ice										
Marijuana										
Alcohol										
Cigarette										
Valium										
Midazolam										
Flunitrazepam										
Cough mixture										
Solvents										
Others										

	Column 1 (In Hong Kong)	Column 2 (place specified in Q20)

28. On each occasion, how much do you spend to buy drugs?

	$_____	$_____
☐ Free	☐	☐

	Column 1 (In Hong Kong)	Column 2 (place specified in Q20)

29. Where did you obtain the drugs? (You can check more than one box)

1)	Friends	☐	☐
2)	Friends in Triad societies	☐	☐
3)	Classmates	☐	☐
4)	Pharmacy	☐	☐
5)	Dealers at cyber cafés	☐	☐
6)	Dealers at discos or rave parties	☐	☐
7)	Family	☐	☐
8)	Others; please specify : _____	☐	☐

30. Apart from rave parties and discos, what other locations do you take drugs? (You can check more than one box)

1)	At home	☐	☐
2)	Friend's house	☐	☐
3)	Street corner	☐	☐
4)	Cyber café	☐	☐
5)	Park	☐	☐
6)	Public toilet	☐	☐
7)	Hotel/Motel	☐	☐
8)	Others; please specify : _____	☐	☐

31. Have you ever been sent to a hospital because of drug overdose?

1)	Yes	☐	☐
2)	Never	☐	☐

First time drug use

32. Have you ever tried at least one of the drugs listed in Q27c (excluding cigarette and alcohol)?
    1) ☐ Yes. What was the first drug you tried? _____
    2) ☐ No, (Go to Q61)

33. How long ago did you first take this drug? _____(Yr)_____(Mth) ago

34. Where did you obtain the drug? (You can check more than one box)
    1) ☐ Friends
    2) ☐ Friends in Triad society
    3) ☐ Classmates
    4) ☐ Pharmacy
    5) ☐ Dealers in cyber cafés
    6) ☐ Dealers in disco or rave party
    7) ☐ I brought back from outside Hong Kong
    8) ☐ Family
    9) ☐ Others; please specify : _____

35. After you first-take this drug, what is the frequency of subsequent use?
    1) ☐ Always
    2) ☐ Often
    3) ☐ Seldom
    4) ☐ Never, go to Q37

36. How frequent will you take this drug?
    1) ☐ Once a day
    2) ☐ 1 to 6 times a week
    3) ☐ 1 to 3 times a month
    4) ☐ Less than once a month
    5) ☐ No fix time

37. The reasons you take drugs for the first time:
(You can check more than one box)

1) ☐ To feel more mature, and to show off to others
2) ☐ To seek excitement and fun
3) ☐ Peer pressure
4) ☐ To be trendy
5) ☐ To relieve boredom/depression
6) ☐ Curiosity
7) ☐ To avoid personal problems
8) ☐ To relax and relieve anxiety
9) ☐ To relieve fatigue and increase alertness
10) ☐ Others; please specify:_____

The reasons of drug taking

38. Which drug do you use most frequently? _____

39. Have you ever taken more than one drug at a time?

1) ☐ Yes Which drugs do you take, rank order according to number of times used?

1.1) _____
1.2) _____
1.3) _____

2) ☐ No

40. When was the last time you take drug? _____(Yr)_____(Mth)

41a. How did you finance your [Drug] use?

1) ☐ Mainly by income from employment.
2) ☐ Mainly by borrowing from family members.
3) ☐ Mainly by borrowing from friends.
4) ☐ Mainly by committing crimes.
5) ☐ A combination of income and borrowing.
6) ☐ A combination of income and committing crimes.
7) ☐ A combination of borrowing and committing crimes.
8) ☐ Others; please specify:_____

41b. What is your average monthly income? $_____

42. What is your monthly expenditure in [drugs] use? $_____

43. Please state whether the drug(s) in Q38 that you use most frequently create the following effects after you take them? (You can check more than one box)

1) ☐ Make yourself feel better when down or depressed
2) ☐ Help you stop worrying about a problem
3) ☐ Help you to relax
4) ☐ Help you feel elated or euphoric
5) ☐ Just get really stoned or intoxicated
6) ☐ Enhance feelings when having sex
7) ☐ Help you to stay awake
8) ☐ Help you lose weight
9) ☐ Help you to sleep
10) ☐ Help you enjoy the company of your friends
11) ☐ Help you feel more confident or more able to talk to people in a social situation
12) ☐ Help you lose your inhibitions
13) ☐ Help you keep going on a night out with friends
14) ☐ Help you to concentrate or to work or study
15) ☐ Enhance an activity such as listening to music or playing a game or sport
16) ☐ Help make something you were doing less boring
17) ☐ Improve the effects of other substances
18) ☐ Help ease the after effects of other substances

The negative effects of drug use

(After taking the drugs in Q38, have you found any following symptoms with 24 hours?)

44a. Physical signs

1) ☐ Increase or decrease in appetite
2) ☐ Staggering walk; poor physical coordination
3) ☐ Inability to sleep
4) ☐ Sweaty palms; shaking hands
5) ☐ Dizzy; headache
6) ☐ Irregular heartbeat
7) ☐ Nausea, vomiting
8) ☐ Extreme hyperactivity, e.g. Dancing

44b. Behavioral signs

1) ☐ Amnesia
2) ☐ Hallucinogens 2.1) ☐ hearing 2.2) ☐ seeing 2.3) ☐ smelling
3) ☐ Feeling depressed or uninterested in things more than 24 hours
4) ☐ Feeling suspicious or paranoid of people more than 24 hours
5) ☐ Negatively affected your family relationships and friendships
6) ☐ Negatively affected your job or school performance
7) ☐ Jumpy or nervous more than 24 hours
8) ☐ Twitch
9) ☐ Become violent
10) ☐ Suicide attempt

Potential tolerance/ dependence

45. Have you spent a great deal of time in those activities necessary to obtain the drugs or use the drugs, e.g. in a period of a month or above? ☐ Yes ☐ No

46. Have you often wanted to or tried to cut down on drugs? ☐ Yes ☐ No

47. Did you ever find you could not stop or cut down using drugs? ☐ Yes ☐ No

48a. Did you ever need markedly increased amounts of drugs to get an effect or find that you could no longer get high on the amount you used to use? ☐ Yes ☐ No

48b. Would you say 50% more? ☐ Yes ☐ No

49. Have you often given up or greatly reduced important activities with friends or relatives or at work in order to use Drugs? ☐ Yes ☐ No

50. Have you often use drugs more days or in larger amounts than you intended to do? ☐ Yes ☐ No

51. Has stopping, cutting down on, or quitting Drug ever caused you any of these problems?
(You can check more than one box)

		Yes	No
1)	Feel depressed	☐	☐
2)	Feel nervous or restless	☐	☐
3)	Feel tired or sleepy	☐	☐
4)	Have trouble sleeping	☐	☐
5)	Increase or decrease in appetite	☐	☐
6)	Tremble or twitching	☐	☐
7)	Sweating or fever	☐	☐
8)	Nausea or vomiting	☐	☐
9)	Diarrhea or stomachaches	☐	☐
10)	Watery eyes or runny nose	☐	☐
11)	Yawning	☐	☐
12)	Increase in heart rate	☐	☐

52. Was there a time when two or more of these symptoms in Q44 occurred together because you were not using [Drug] in Q51? ☐ Yes ☐ No

53. Have you often used Drugs in Q38 to make these withdrawal symptoms go away or to keep from having them? ☐ Yes ☐ No

54a. Did using Drugs cause you to have any other physical health problems; other than withdrawal?

1) ☐ If yes; please specify: _____

2) ☐ No (Go to Q55a)

54b. Did you continue to use Drugs after you knew it caused this problem? ☐ Yes ☐ No

55a. Did you ever experience objections from family, friends, boss or people at work or school because of your Drug use? ☐ Yes ☐ No (Go to Q56)

55b. Did you continue to use Drug after you realized it was causing a problem? ☐ Yes ☐ No

56. Have you often been high on drugs or suffering its after-effects while in school working or taking care of household responsibilities? ☐ Yes ☐ No

57a. While using [drugs], did you ever have any psychological problems start or get worse, such as feeling depressed feeling paranoid, trouble thinking clearly, hearing, smelling, or seeing things or feeling jumpy?

1) ☐ If yes, please specify which problem(s)

1.1) _____

1.2) _____

1.3) _____

2) ☐ No, got to Q58

57b. Did you continue to use [drugs] after you knew it cause any of these problem? ☐ Yes ☐ No

58. Have you often been under the effects of Drug in a situation where it increased your chances of getting hurt-for instance, when driving, using knives or machinery, crossing against traffic, climbing or swimming? ☐ Yes ☐ No

59. Have you ever received any treatment for using drugs of abuse?

1) ☐ If yes, are you still receiving treatment?

1.1) ☐ Yes

1.2) ☐ No

2) ☐ No

60. Do you have any criminal records?

1) ☐ If yes, does the record relate to drug?

1.1) ☐ Yes

1.2) ☐ No

1.3) ☐ Both have tried

2) ☐ No

Knowledge about drugs of abuse

61. Please state 3 drugs you know best:
    1) _____ 2) _____ 3) _____

62. Where did you get to know about these drugs?
    1) ☐ TV/Radio/Movie
    2) ☐ Friends
    3) ☐ Social worker
    4) ☐ Newspaper
    5) ☐ Parents
    6) ☐ School
    7) ☐ Others; please specify:_____

63. Do you think that the aforementioned drugs can cause harm to your body? ☐ Yes ☐ No

64. Do you think that the aforementioned drugs can cause mental problems? ☐ Yes ☐ No

65. Do you think that the aforementioned drugs are addictive and may lead to dependence? ☐ Yes ☐ No

66. Do you think some of the drugs that you take may be contaminated with other drugs? ☐ Yes ☐ No

67. Do you think that the aforementioned drugs can cause permanent damage to your body? ☐ Yes ☐ No

68. Do you think that the taking of the aforementioned drugs can result in death? ☐ Yes ☐ No

69. Do you think the using of needles to inject aforementioned drugs can cause AIDS? ☐ Yes ☐ No

Psychological well being

70. How stressful is your daily life?
    1) ☐ Very stressful
    2) ☐ Quite stressful
    3) ☐ A little stressful
    4) ☐ Not stressful at all

71. Do you feel that you have control over your own life?
    1) ☐ In complete control
    2) ☐ Partly in control
    3) ☐ Undecided
    4) ☐ Partly lost control
    5) ☐ Completely lost control

72 In general, are you satisfied with your life?
    1) ☐ Very satisfied
    2) ☐ Satisfied
    3) ☐ Undecided
    4) ☐ Dissatisfied
    5) ☐ Very dissatisfied

The End, Thank you very much!

Appendix 2a Chinese version of SSS-V scale

訪問員：_____ 訪問日期：_____ 問卷編號：_____

以下每題包括兩個選擇: 甲，乙。請圈上那個最能形容你的感受之選擇。若兩個選擇皆不能貼切地形容你的感受，請選擇一個比較近似的形容。
請於每題只選擇 (圈)一個答案。
請表達你真實的感受，不要介意別人的看法，這裡並沒有對或錯的答案。

1. 甲 我喜歡狂野，不受限制的派對。
   乙 我比較喜歡安靜，可以好好談話的派對。
2. 甲 有些電影，我會看上二至三次。
   乙 我不能忍受看同一套電影超過一遍。
3. 甲 我常渴望可以成爲攀山者。
   乙 我不能理解那些冒生命危險攀山的人。
4. 甲 我討厭所有身體氣味。
   乙 我喜歡一些肉體的氣味。
5. 甲 我看見一些舊面孔會感到沉悶。
   乙 我享受朋友給我的親切感。
6. 甲 即使要付上代價，我也喜歡獨自去探索一個城市或地方。
   乙 在一個我不大熟悉的環境，我希望有一個導遊。
7. 甲 我討厭有些人有意做/說一些東西去嚇驚或觸怒別人。
   乙 當你可以預計某人將會做/說一些東西，他一定是一個悶旦。
8. 甲 我通常不會享受一套我會預計到劇情的電影或戲劇。
   乙 即使我可以預計到劇情，我也不介意看那套電影或戲劇。
9. 甲 我嘗試過或希望嘗試食大麻。
   乙 我永不會服食大麻。
10. 甲 我不會嘗試會產生怪異或危險效果的藥物。
    乙 我希望嘗試會產生幻覺的新藥。
11. 甲 一個正常的人會免危險的活動。
    乙 我有時喜歡做帶有少許危險的活動。
12. 甲 我討厭性開放的人。
    乙 我喜歡與性開放的人罵伍。
13. 甲 我覺得興奮劑令我感到不舒服。
    乙 我常喜歡感到情緒高漲。(飲用酒精或吸食大麻)
14. 甲 我喜歡嘗試新食物。
    乙 爲了避免失望，我點菜時會選擇一些熟悉的食物。
15. 甲 我喜歡看家庭錄影帶或旅遊放映片。
    乙 看別人的家庭錄影帶或旅遊放映片，令我感到非常沉悶。
16. 甲 我希望學習滑水。
    乙 我不會希望學習滑水。
17. 甲 我希望嘗試滑浪。
    乙 我不會希望嘗試滑浪。
18. 甲 去旅遊時我不喜歡預先安排或特定行程/時間表。
    乙 當我參加旅遊時，我喜歡預先小心計劃行程/時間表。

19. 甲 我比較喜歡與踏實型的人爲伍。
乙 我喜歡與一些不平凡(如藝術家或嬉皮士)的人爲伍。
20. 甲 我不會希望學習駕駛飛機。
乙 我希望學習駕駛飛機。
21. 甲 我喜歡水面多於深水處。
乙 我喜歡到深海潛水。
22. 甲 我喜歡認識一些同性戀者(男或女)。
乙 我會避免與一些懷疑同性戀的人交往。
23. 甲 我想嘗試跳降落傘。
乙 不論有沒有降落傘，我永不會渴望由飛機跳出來。
24. 甲 我比較喜歡那些難以預測，給人帶來刺激的朋友。
乙 我比較喜歡那些容易預測及可靠的朋友。
25. 甲 我沒有興趣爲經歷而經歷。
乙 我喜歡新和刺激的經驗和感覺，即使它們有些危險，反傳統或違法。
26. 甲 藝術的精髓在於其清晰，對稱及和諧的色調。
乙 我覺得撞色和不規則的現代畫很有美感。
27. 甲 我享受在家熟悉的環境。
乙 要我在家待一些時間，我感到忐忑不安。
28. 甲 我喜歡高台跳水。
乙 我不喜歡站在高台的感覺。(或我不會走近它)
29. 甲 我喜歡約會身材吸引的異性。
乙 我喜歡約會能分享價值觀的異性。
30. 甲 豪飲通常會破壞一個派對，因爲有些人會很嘈吵及瘋狂。
乙 一個好的派對絕不缺少豪情暢飲。
31. 甲 無禮是社交的大忌。
乙 沉悶是社交的大忌。
32. 甲 一個人結婚前應該有好些性經驗。
乙 兩夫婦在婚後開始與對方發生性關係會此較好。
33. 甲 即使我有錢，我也不會與一些隨便揮霍的人爲伍。
乙 我相信我會與一些富家子弟到處尋歡。
34. 甲 我喜歡一些聰明和機智的人，即使他們會有時侮辱他人。
乙 我討厭一些人將自己的快樂建築在別人的痛苦上。
35. 甲 在電影中實在太多關於性的描述。
乙 我喜歡電影中的"性感"場面。
36. 甲 當我飲了幾杯酒時，我的感覺是最好的。
乙 那些要靠酒精帶來快樂的人很有問題。
37. 甲 人們的衣著應該以品味和整齊爲標準。
乙 人們應該擁有個人的衣著風格，即使效果有時比較怪異。
38. 甲 用小船航行一段長的路程是一件愚蠢的事。
乙 我希望乘坐一隻可以越洋的小船去很遠的地方。
39. 甲 我對平凡或沉悶的人缺乏耐性。
乙 我幾乎在所有人身上都找到有趣的地方。
40. 甲 從山坡上快速滑下必定重傷。
乙 我相信我會享受由山坡高處快速滑下的感覺。

Appendix 2b Chinese version of BIS/BAS scale

訪問員：_____ 訪問日期：_____ 問卷編號：_____

請細閱此問卷中每一項，用 1，2，3，4 代表你有多同意該項。所有項目必須回答。請獨立地考慮每項，毋需擔心不同項目之間答案不一致，只要準確及誠實地回答便可。(請在適當的方格上填"√")

1 = 非常同意　　2 = 有點同意　　3 = 有點不同意　　4 = 非常不同意

		1	2	3	4
1	家庭在一個人的人生中是最重要的。	□	□	□	□
2	即使有壞的事情發生在我身上，我也很少感到恐懼或緊張。	□	□	□	□
3	我會竭盡所能去得到我想要的東西。	□	□	□	□
4	當我把事情做得好時，我會喜歡繼續去做它。	□	□	□	□
5	我總是願意嘗試我覺得可能會是有趣的新事物。	□	□	□	□
6	我注重個人的衣著。	□	□	□	□
7	當我得到我想要的東西時，我便感到興奮及充滿活力。	□	□	□	□
8	被批評或被責罵對我造成較嚴重的傷害。	□	□	□	□
9	我通常會盡全力去追求我渴望得到的東西。	□	□	□	□
10	我常常會去做某些事，只因爲它們有趣。	□	□	□	□
11	對於某些要做的事，例如剪頭髮，我感到難以抽空去做。	□	□	□	□
12	當我知道有機會去得到我想要的東西時，我會立即採取行動。	□	□	□	□
13	當我認爲或知道有人生我的氣，我會感到非常擔憂及苦惱。	□	□	□	□
14	當我遇到一個機會能得到我喜歡的東西時，我會立即興奮起來。	□	□	□	□
15	我常因一時興奮就行動。	□	□	□	□
16	若我覺得有不愉快的事情要發生，我通常會坐立不安。	□	□	□	□
17	我經常想知道爲何人們會有這樣的舉動。	□	□	□	□
18	當有如意的事情發生在我身上，我會受到強烈的影響。	□	□	□	□
19	我會因爲弄垮了重要的事情而感到憂心。	□	□	□	□
20	我渴求獲得刺激及新感覺。	□	□	□	□
21	我會以"排除萬難"的態度去追求我渴望得到的東西。	□	□	□	□
22	相比我的朋友我很少有憂慮的情緒。	□	□	□	□
23	當我贏得比賽時，我覺得很興奮。	□	□	□	□
24	我擔心我犯錯。	□	□	□	□